

**From:** Mandsager, Kathy  
**Location:** via WebEx (instructions below)  
**Importance:** Normal  
**Subject:** State-of-science: Degradation & Fate Group  
**Start Date/Time:** Mon 1/30/2017 8:00:00 PM  
**End Date/Time:** Mon 1/30/2017 10:00:00 PM  
2017.01.05 Degradation and Fate.docx

This is just a reminder of our call scheduled for Monday beginning at 3:00 pm ET. Attached is the latest version of our document.

### **State-of-Science: Degradation & Fate**

Monday, January 30, 2017

3:00 pm | Eastern Standard Time (New York, GMT-05:00) | 2 hrs

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**From:** Mandsager, Kathy  
**Location:** via WebEx (login instructions below)  
**Importance:** Normal  
**Subject:** State-of-Science for Dispersant Use in Arctic Waters DEGRDATION & FATE group  
**Start Date/Time:** Wed 12/14/2016 7:00:00 PM  
**End Date/Time:** Wed 12/14/2016 9:00:00 PM  
2016.11.28 Degradation and Fate.docx

This is a reminder that we have TWO meetings scheduled this week: tomorrow/Wednesday 12/14 AND Thursday 12/15; both begin at 2:00 pm ET. The WebEx login instructions are below and attached is our latest version of the document for your preparation in the discussion.

### **Degradation & Fate group**

Wednesday, December 14, 2016

2:00 pm | Eastern Standard Time (New York, GMT-05:00) | 2 hrs

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**From:** Mandsager, Kathy  
**Location:** via WebEx instructions below  
**Importance:** Normal  
**Subject:** FW: Degradation & Fate science panel meeting  
**Start Date/Time:** Mon 11/28/2016 6:00:00 PM  
**End Date/Time:** Mon 11/28/2016 8:00:00 PM  
2016.10.21 Degradation and Fate.docx  
2016.10.24 Forwarded to Degradation group from public comment to Transport group.docx

Just checking you guys got this. V

-----Original Appointment-----

**From:** Mandsager, Kathy [mailto:kathy.mandsager@unh.edu]  
**Sent:** Thursday, September 08, 2016 9:01 AM  
**To:** Mandsager, Kathy; Conmy, Robyn; fingasmerv@shaw.ca; tchazen@utk.edu;  
robert.jones@noaa.gov; mandyjoye@gmail.com; mbleigh@alaska.edu;  
karl.linden@colorado.edu; kmmcfarlin@alaska.edu; msmls@lsu.edu;  
thomas.s.coolbaugh@exxonmobil.com; mathijs.smit@shell.com; Sprenger, Mark;  
mjoye@uga.edu; Terry Hazen; Gary Shigenaka - NOAA Federal; doug.helton@noaa.gov;  
Principe, Vanessa; Wilson, Gregory  
**Subject:** Degradation & Fate science panel meeting  
**When:** Monday, November 28, 2016 1:00 PM-3:00 PM (UTC-05:00) Eastern Time (US & Canada).  
**Where:** via WebEx instructions below

This is just a reminder of our call today at 1 ET.

### **Degradation & Fate science panel meeting**

Monday, November 28, 2016

1:00 pm | Eastern Standard Time (New York, GMT-05:00) | 2 hrs

Meeting number (access code): 733 596 974

**Add to Calendar**

When it's time, [join the meeting](#).

### **Join by phone**

**1-855-244-8681** Call-in toll-free number (US/Canada)

**1-650-479-3207** Call-in toll number (US/Canada)

[Global call-in numbers](#) | [Toll-free calling restrictions](#)

[Can't join the meeting?](#)

IMPORTANT NOTICE: Please note that this WebEx service allows audio and other information sent during the session to be recorded, which may be discoverable in a legal matter. By joining this session, you automatically consent to such recordings. If you do not consent to being recorded, discuss your concerns with the host or do not join the session.

**From:** Mandsager, Kathy  
**Location:** via Webex (instructions below)  
**Importance:** Normal  
**Subject:** State of Science: Degradation & Fate  
**Start Date/Time:** Thur 8/18/2016 6:00:00 PM  
**End Date/Time:** Thur 8/18/2016 8:00:00 PM  
2016.06.25 LINDEN Degradation and Fate with public input.docx

Reminder of our meeting scheduled for tomorrow at 2pm ET. [Since Karl Linden is on sabbatical this summer and unable to participate in call tomorrow, the attached document includes some of his comments].

### **Degradation & Fate group**

Thursday, August 18, 2016

2:00 pm | Eastern Daylight Time (New York, GMT-04:00) | 2 hrs

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**From:** Kinner, Nancy  
**Location:** via WebEx (instructions below)  
**Importance:** Normal  
**Subject:** State Of Science: Transport & Behavior group  
**Start Date/Time:** Tue 7/19/2016 8:00:00 PM  
**End Date/Time:** Tue 7/19/2016 10:00:00 PM  
2016.06.17 state-of-science on Physical Transport and Chemical Behavior AK.docx

This is just a reminder of our meeting scheduled for tomorrow at 4ET. Attached is the latest version of the document.

**state-of-science: Transport & Behavior group**

Tuesday, July 19, 2016

4:00 pm | Eastern Daylight Time (New York, GMT-04:00) |  
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**To:** Conmy, Robyn[Conmy.Robyn@epa.gov]  
**From:** Zhang, Yu (zhang4y5)  
**Sent:** Wed 7/6/2016 11:41:14 PM  
**Subject:** Answer to the removal extent question in thesis defence  
[Explanation of heavy alkanes removal extents.docx](#)

Hello! Dr. Conmy,

Thanks to the question you asked in my denfence, regarding to the removal extents of heavy alkanes. It is a really good point and I think I find the most possible reason.

The initial concentration of those heavy alkanes are much lower than lighter alkanes, and it is very close to our lowest calibration point (0.05 mg/L) on GC/MS/MS. Therefore, the removal amount of those long chain compounds are lower, but they are easier to reach the undetectable level which resulted in the 100% removal extents. Additionally, I also double checked the shape of the peaks, the integration, and the raw data. Mobing also plotted the similar result in her phase I experiment. The supporting table and figures have been attached.

If you have any other questions, we can also have a few minutes discussion after the meeting tomorrow.

Thanks a lot.

Best Regards,

Yu

**To:** Conmy, Robyn[Conmy.Robyn@epa.gov]; fingasmerv@shaw.ca[fingasmerv@shaw.ca];  
tchazen@utk.edu[tchazen@utk.edu]; robert.jones@noaa.gov[robert.jones@noaa.gov];  
mandyjoye@gmail.com[mandyjoye@gmail.com]; mbleigh@alaska.edu[mbleigh@alaska.edu];  
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**Cc:** nancy.kinner@unh.edu[nancy.kinner@unh.edu]  
**From:** Mandsager, Kathy  
**Sent:** Tue 12/15/2015 7:31:25 PM  
**Subject:** RE: Degradation Group appendix review - REPLY needed  
[2015.12.10 Degradation and Fate FINAL DRAFT.docx](#)

This is just a friendly reminder of our meeting scheduled for tomorrow beginning at 4:00 pm ET.

**From:** Mandsager, Kathy  
**Sent:** Wednesday, December 02, 2015 9:31 AM  
**Subject:** RE: Degradation Group appendix review - REPLY needed

CONFIRMED: our next meeting will be **Wednesday 16 December at 4:00 pm EST** via WebEx (instructions below). I will also send this information as a calendar request for your convenience.

I am also attaching:

- A slightly revised publications review summary sheet
- The published paper (resending previous paper) by Kleindienst and Joye “Chemical dispersants can suppress the activity of natural oil-degrading microorganisms”
- The data supporting this paper – per special request for this group’s in-depth review

**State-of-Science: Degradation & Fate group**

Wednesday, December 16, 2015

4:00 pm | Eastern Standard Time (New York, GMT-05:00) | 2 hrs

## Join WebEx meeting

Meeting number: 739 325 205

### Join by phone

**1-855-244-8681** Call-in toll-free number (US/Canada)

**1-650-479-3207** Call-in toll number (US/Canada)

Access code: 739 325 205

[Global call-in numbers](#) | [Toll-free calling restrictions](#)

**From:** Mandsager, Kathy

**Sent:** Wednesday, November 25, 2015 3:32 PM

**To:** 'conmy.robbyn@epa.gov' <[conmy.robbyn@epa.gov](mailto:conmy.robbyn@epa.gov)>; 'fingasmerv@shaw.ca' <[fingasmerv@shaw.ca](mailto:fingasmerv@shaw.ca)>; 'tchazen@utk.edu' <[tchazen@utk.edu](mailto:tchazen@utk.edu)>; 'robert.jones@noaa.gov' <[robert.jones@noaa.gov](mailto:robert.jones@noaa.gov)>; 'mandyjoye@gmail.com' <[mandyjoye@gmail.com](mailto:mandyjoye@gmail.com)>; 'mbleigh@alaska.edu' <[mbleigh@alaska.edu](mailto:mbleigh@alaska.edu)>; 'karl.linden@colorado.edu' <[karl.linden@colorado.edu](mailto:karl.linden@colorado.edu)>; 'kmmcfarlin@alaska.edu' <[kmmcfarlin@alaska.edu](mailto:kmmcfarlin@alaska.edu)>; 'msmiles@lsu.edu' <[msmiles@lsu.edu](mailto:msmiles@lsu.edu)>; 'thomas.s.coolbaugh@exxonmobil.com' <[thomas.s.coolbaugh@exxonmobil.com](mailto:thomas.s.coolbaugh@exxonmobil.com)>; 'mathijs.smit@shell.com' <[mathijs.smit@shell.com](mailto:mathijs.smit@shell.com)>; 'sprenger.mark@epa.gov' <[sprenger.mark@epa.gov](mailto:sprenger.mark@epa.gov)>

**Cc:** Kinner, Nancy <[Nancy.Kinner@unh.edu](mailto:Nancy.Kinner@unh.edu)>

**Subject:** RE: Degradation Group appendix review - REPLY needed

Degradation Group members,

Thank you for those who reviewed and submitted your suggestions on the additional papers that



were put forth for your review. Please find attached the tally sheet of these comments. We now like to schedule one more meeting to have a final discussion on the following items:

1. The consensus of these additional papers
2. Confirm the statement in our paper (line 311)
3. Confirm if the “areas of disagreements stand (line 348)
4. Discuss the recently published paper by Kleindienst and Joye “Chemical dispersants can suppress the activity of natural oil-degrading microorganisms”

Please use this doodle poll (by 12/2) to select our next (hopefully brief) conference call (12/10 to 12/16) >> <http://doodle.com/poll/yzvfztmu82u9md4n>. this poll is time zone enabled for your convenience.

Happy Thanksgiving!

**From:** Mandsager, Kathy  
**Sent:** Thursday, October 29, 2015 1:40 PM  
**Subject:** RE: Degradation Group appendix review

REMINDER: Comments on these additional papers are due tomorrow. Per the results, I will be scheduling another call to discuss.

Thank you!

**From:** Mandsager, Kathy  
**Sent:** Monday, September 28, 2015 5:31 PM  
**To:** 'conmy.robbyn@epa.gov' <[conmy.robbyn@epa.gov](mailto:conmy.robbyn@epa.gov)>; 'fingasmerv@shaw.ca' <[fingasmerv@shaw.ca](mailto:fingasmerv@shaw.ca)>; 'tchazen@utk.edu' <[tchazen@utk.edu](mailto:tchazen@utk.edu)>; 'robert.jones@noaa.gov' <[robert.jones@noaa.gov](mailto:robert.jones@noaa.gov)>; 'mandyjoye@gmail.com' <[mandyjoye@gmail.com](mailto:mandyjoye@gmail.com)>; 'mbleigh@alaska.edu' <[mbleigh@alaska.edu](mailto:mbleigh@alaska.edu)>; 'karl.linden@colorado.edu' <[karl.linden@colorado.edu](mailto:karl.linden@colorado.edu)>; 'kmmcfarlin@alaska.edu' <[kmmcfarlin@alaska.edu](mailto:kmmcfarlin@alaska.edu)>;

'msmiles@lsu.edu' <msmiles@lsu.edu>; 'thomas.s.coolbaugh@exxonmobil.com' <thomas.s.coolbaugh@exxonmobil.com>; 'mathijs.smit@shell.com' <mathijs.smit@shell.com>; 'sprenger.mark@epa.gov' <sprenger.mark@epa.gov>  
**Cc:** Kinner, Nancy <Nancy.Kinner@unh.edu>; 'Ian Gaudreau' <iangaudreau@gmail.com>; Mandsager, Kathy <kathy.mandsager@unh.edu>  
**Subject:** Degradation Group appendix review

Degradation Group,

As follow-up to our call on Friday, a list of publications for your review are located here>>><https://unh.box.com/s/wwn2juyzfkgt5bd20n4s8e8bo3uq5t93>.

To make it easier for your review, we have a spreadsheet (attached) for your input. Please simple say “yes” or “no” in each of the 2 columns next to each publication. Share any comments in order to clarify or support your vote on each publication.

Please submit by **Friday 30 October**.

---

Kathy Mandsager

Program Coordinator

Coastal Response Research Center

Center for Spills and Environmental Hazards

234 Gregg Hall, Colovos Rd

University of New Hampshire

Durham, NH 03824

603.862.1545

# Chemical dispersants can suppress the activity of natural oil-degrading microorganisms

Sara Kleindienst<sup>a,1</sup>, Michael Seidel<sup>a,2</sup>, Kai Ziervogel<sup>b</sup>, Sharon Grim<sup>c,3</sup>, Kathy Loftis<sup>a,4</sup>, Sarah Harrison<sup>a</sup>, Sairah Y. Malkin<sup>a</sup>, Matthew J. Perkins<sup>d</sup>, Jennifer Field<sup>d</sup>, Mitchell L. Sogin<sup>c</sup>, Thorsten Dittmar<sup>e,f</sup>, Uta Passow<sup>g</sup>, Patricia M. Medeiros<sup>a</sup>, and Samantha B. Joye<sup>a,5</sup>

<sup>a</sup>Department of Marine Sciences, University of Georgia, Athens, GA 30602; <sup>b</sup>Department of Marine Sciences, University of North Carolina, Chapel Hill, NC 27599; <sup>c</sup>Josephine Bay Paul Center, Marine Biological Laboratory, Woods Hole, MA 02543; <sup>d</sup>Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR 97331; <sup>e</sup>Research Group for Marine Geochemistry, Institute for Chemistry and Biology of the Marine Environment (ICBM), Carl von Ossietzky University, 26129 Oldenburg, Germany; <sup>f</sup>Max Planck Institute for Marine Microbiology (MPI), 28359 Bremen, Germany; and <sup>g</sup>Marine Science Institute, University of California, Santa Barbara, CA 93106

Edited by William H. Schlesinger, Cary Institute of Ecosystem Studies, Millbrook, NY, and approved September 25, 2015 (received for review April 15, 2015)

During the Deepwater Horizon oil well blowout in the Gulf of Mexico, the application of 7 million liters of chemical dispersants aimed to stimulate microbial crude oil degradation by increasing the bioavailability of oil compounds. However, the effects of dispersants on oil biodegradation rates are debated. In laboratory experiments, we simulated environmental conditions comparable to the hydrocarbon-rich, 1,100 m deep plume that formed during the Deepwater Horizon discharge. The presence of dispersant significantly altered the microbial community composition through selection for potential dispersant-degrading *Colwellia*, which also bloomed in situ in Gulf deep waters during the discharge. In contrast, oil addition to deep water samples in the absence of dispersant stimulated growth of natural hydrocarbon-degrading *Marinobacter*. In these deepwater microcosm experiments, dispersants did not enhance heterotrophic microbial activity or hydrocarbon oxidation rates. An experiment with surface seawater from an anthropogenically derived oil slick corroborated the deep water microcosm results as inhibition of hydrocarbon turnover was observed in the presence of dispersants, suggesting that the microcosm findings are broadly applicable across marine habitats. Extrapolating this comprehensive dataset to real world scenarios questions whether dispersants stimulate microbial oil degradation in deep ocean waters and instead highlights that dispersants can exert a negative effect on microbial hydrocarbon degradation rates.

oceanography | microbial dynamics | hydrocarbon cycling | chemical dispersants | oil spills

Crude oil enters marine environments through geophysical processes at natural hydrocarbon seeps (1) at a global rate of ~700 million liters per year (2). In areas of natural hydrocarbon seepage, such as the Gulf of Mexico (hereafter, the Gulf), exposure of indigenous microbial communities to oil and gas fluxes can select for microbial populations that use petroleum-derived hydrocarbons as carbon and energy sources (3, 4). The uncontrolled deep-water oil well blowout that followed the explosion and sinking of the Deepwater Horizon (DWH) drilling rig in 2010 released about 750 million liters of oil into the Gulf. Seven million liters of chemical dispersants were applied (5) with the goal of dispersing hydrocarbons and stimulating oil biodegradation. A deep-water (1,000–1,300 m) plume, enriched in hydrocarbons (6–11) and dioctyl sodium sulfosuccinate (DOSS) (12, 13), a major component of chemical dispersants (14), formed early in the discharge (7). The chemistry of the hydrocarbon plume significantly altered the microbial community (11, 15–17), driving rapid enrichment of low-abundance bacterial taxa such as *Oceanospirillum*, *Cycloclasticus*, and *Colwellia* (18). The natural hydrocarbon degraders in Gulf waters were either in low abundance or absent in DWH deep-water plume samples (18).

Chemical dispersants emulsify surface oil slicks, reduce oil delivery to shorelines (19), and increase dissolved oil concentrations, which should make oil more bioavailable (20) and stimulate

biodegradation (21). The efficacy of dispersants in stimulating oil biodegradation is debated (22) and negative environmental effects have been documented (23). Dispersant application often requires ecological tradeoffs (24). Surprisingly little is known about the impacts of dispersants on the activity and abundance of hydrocarbon-degrading microorganisms (25). This work addressed three key questions: (i) Do dispersants influence microbial community composition? (ii) Is the indigenous microbial community as effective at oil biodegradation as microbial populations following dispersant/dispersed oil exposure? (iii) Does chemically dispersed oil stimulate hydrocarbon biodegradation rates?

Laboratory experiments were used to unravel the effects of oil-only (supplied as a water-accommodated fraction, “WAF”), Corexit 9500 (“dispersant-only”), oil–Corexit 9500 mixture (chemically enhanced

## Significance

Oil spills are a significant source of hydrocarbon inputs into the ocean. In response to oil spills, chemical dispersants are applied to the oil-contaminated seawater to disperse surface slicks into smaller droplets that are presumed to be more bioavailable to microorganisms. We provide evidence that chemical dispersants applied to either deep water or surface water from the Gulf of Mexico did not stimulate oil biodegradation. Direct measurement of alkane and aromatic hydrocarbon oxidation rates revealed either suppression or no stimulation of oil biodegradation in the presence of dispersants. However, dispersants affected microbial community composition and enriched bacterial populations with the ability to use dispersant-derived compounds as growth substrates, while oil-alone amendments enriched for natural hydrocarbon degraders.

Author contributions: S.K., S.H., S.Y.M., and S.B.J. designed research; S.K., M.S., K.Z., K.L., S.H., S.Y.M., M.J.P., J.F., and U.P. performed research; S.G., K.L., M.J.P., J.F., M.L.S., T.D., and P.M.M. contributed new reagents/analytic tools; S.K., M.S., K.Z., S.G., S.H., S.Y.M., M.J.P., J.F., M.L.S., T.D., U.P., P.M.M., and S.B.J. analyzed data; and S.K., M.L.S., P.M.M., and S.B.J. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

Data deposition: 16S rRNA amplicon Illumina sequencing data were deposited in the GenBank database (BioProject accession no. PRJNA253405).

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<sup>2</sup>Present address: Research Group for Marine Geochemistry, Institute for Chemistry and Biology of the Marine Environment (ICBM), Carl von Ossietzky University, 26129 Oldenburg, Germany; and Max Planck Institute for Marine Microbiology (MPI), 28359 Bremen, Germany.

<sup>3</sup>Present address: Department of Earth and Environmental Sciences, University of Michigan, Ann Arbor, MI 48109.

<sup>4</sup>Present address: Center for Applied Isotope Studies, University of Georgia, Athens, GA 30602.

<sup>5</sup>To whom correspondence should be addressed. Email: mjoye@uga.edu.

This article contains supporting information online at [www.pnas.org/lookup/suppl/doi:10.1073/pnas.1507380112/-DCSupplemental](http://www.pnas.org/lookup/suppl/doi:10.1073/pnas.1507380112/-DCSupplemental).

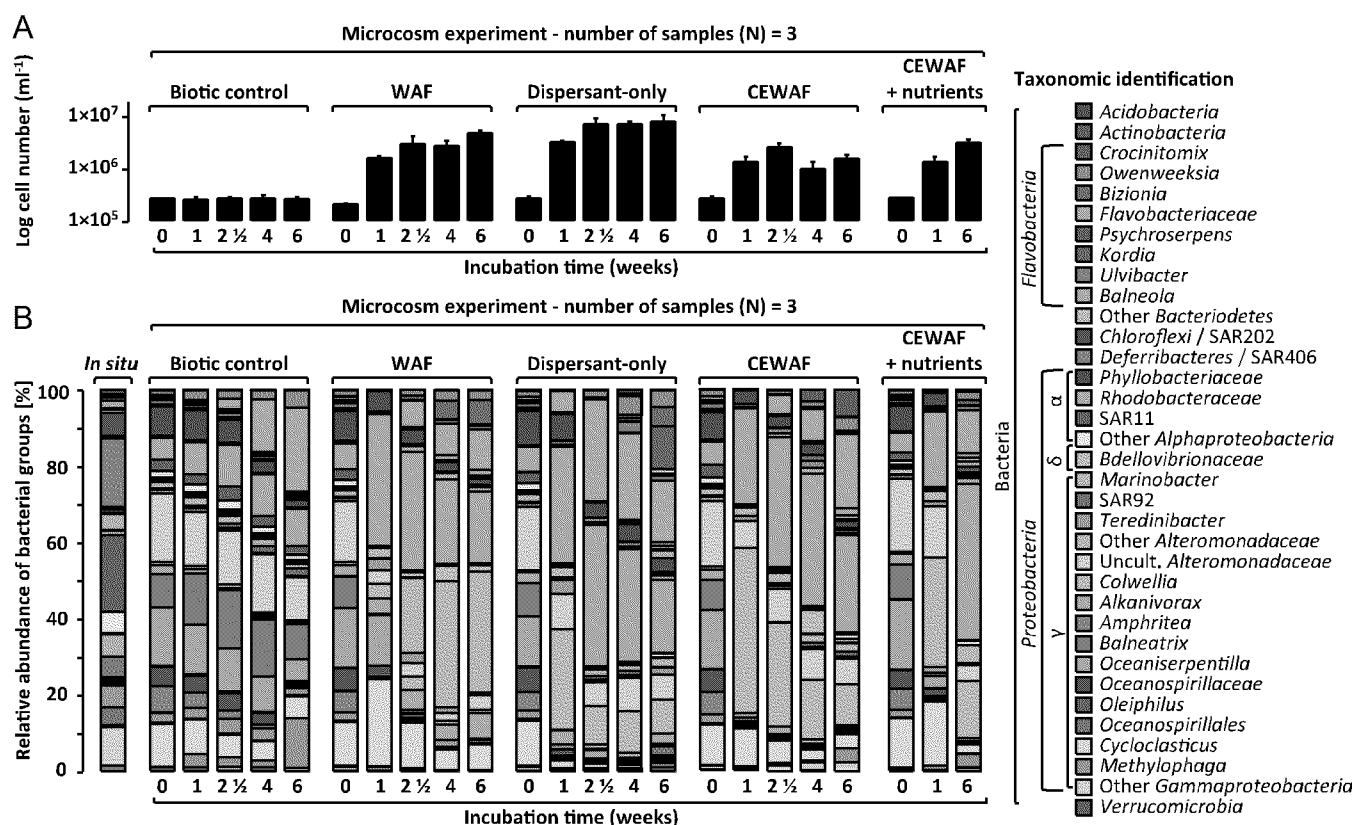


Fig. 1. Dispersants affect the evolution of oil-degrading microbial populations. (A) Average and standard deviation (SD) of cell numbers from sample triplicates (log scale) monitored for 6 wk in microcosms. (B) Relative abundance of bacterial groups in Gulf of Mexico deep water *in situ* samples and in the microcosms (average of triplicate samples). Reads of the V4V5 regions of the 16S rRNA gene were clustered into operational taxonomic units and taxonomy was assigned with Global Alignment for Sequence Taxonomy (GAST).

water-accommodated fraction, CEWAF) or a CEWAF with nutrients (CEWAF + nutrients) (SI Appendix) on Gulf deep-water microbial populations (SI Appendix, SI Text and Figs. S1 and S2). Experimental conditions (SI Appendix, Table S1) mimicked those prevailing in the DWH deep-water hydrocarbon plume (6–13, 18), the chemistry of which varied substantially over space and time (18). Amending samples with WAFs and CEWAFs assured that observed differences in microbial community composition and activity would be driven by compositional differences (e.g., the presence or absence of dispersants) in the dissolved organic carbon (DOC) pool rather than by differences in the bulk DOC concentration (26, 27). We developed an improved radiotracer method to directly quantify hydrocarbon oxidation rates. The microbial community composition was monitored over time using 16S rRNA amplicon sequencing. Dispersant application selected for specific microbial taxa and oligotypes with 16S rRNA gene sequences similar to those recovered *in situ* during the DWH discharge. Surprisingly, CEWAF ( $\pm$  nutrients) addition did not enhance microbial activity or microbial oil-degradation rates.

## Results and Discussion

**Dispersant Significantly Altered Microbial Community Composition.** We hypothesized that dispersants would alter microbial community composition in the deepwater samples and that selection of one population over another would drive differences in hydrocarbon-degradation rates, altering the oil-degradation efficiency. We explored patterns in microbial abundance (Fig. 1A) using microscopy and community composition by Illumina paired-end sequencing of bacterial 16S rRNA gene amplicons (Fig. 1B). We resolved closely related bacterial taxa using oligotyping analysis (28) (Fig. 2 and SI Appendix, Fig. S3). We elucidated the

ecological preference of specific taxa using statistical correspondence analysis (SI Appendix, Figs. S4–S8).

All dispersant-amended treatments showed ingrowth of *Colwellia* (SI Appendix, Fig. S4), a group containing both hydrocarbon and dispersant degraders (29). After 1 wk, the relative abundance of *Colwellia* increased from 1% to 26–43% in dispersant-only and CEWAF ( $\pm$  nutrients) treatments (Fig. 1B). In contrast, *Colwellia* was a minority (1–4%) in WAF treatments. Selective enrichment of *Colwellia* in dispersant-only treatments indicates that dispersant components served as growth substrates (29). The relative abundance of *Colwellia* oligotypes 01, 02, and 05 increased in dispersant treatments (Fig. 2 and SI Appendix, Fig. S5), whereas oligotypes 03 and 10 increased in treatments receiving oil only, underscoring the role of dispersants in driving variation in *Colwellia* taxa. Phylogenetic analysis of the 16S rRNA gene amplicons confirmed that these oligotypes were closely related to species detected in DWH plume samples *in situ* (9, 16, 18) (SI Appendix, Fig. S9), verifying the environmental relevance of these organisms during the DWH discharge.

The dominant microbial responder to WAF addition was *Marinobacter*, whose relative abundance increased from 2% to 42% after 4 wk (Fig. 1B). In contrast, in dispersant-only and CEWAF ( $\pm$  nutrients) treatments, *Marinobacter* comprised only 1–5% of all sequences. The correspondence analysis emphasized the dominance of *Marinobacter* in WAF samples (SI Appendix, Fig. S6) and the same *Marinobacter* oligotypes occurred across all treatments, illustrating that dispersants did not select for specific *Marinobacter* taxa, as was the case for *Colwellia* (SI Appendix, Fig. S3A). *Marinobacter* (SI Appendix, Fig. S10) degrade a wide variety of hydrocarbons, including pristane, hexadecane, octane, toluene, benzynes, and phenanthrene (30–32) and are likely dominant hydrocarbon degraders under natural conditions. However, their abundance clearly declined in the presence of dispersants. Whether

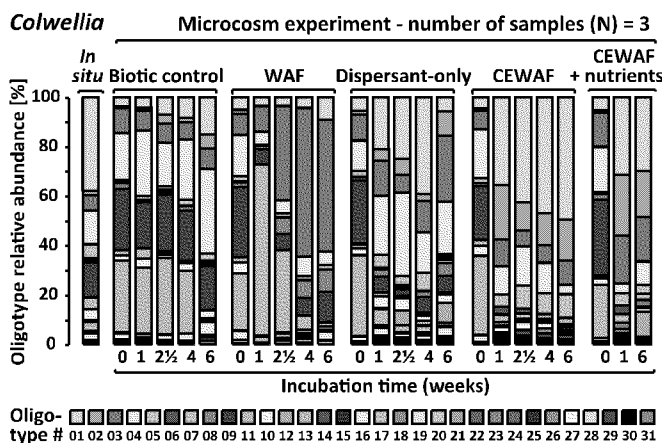


Fig. 2. Different microbial oligotypes respond to dispersants or oil (WAF). Oligotyping enabled the interpretation of 16S rRNA gene sequence diversity at the level of specific oligotypes. Relative abundance (averaged across biological triplicates) of *Colwellia* oligotypes in microcosms, simulating DWH spill-like plumes.

*Colwellia* outcompetes *Marinobacter* or whether *Marinobacter* is inhibited by some component of Corexit 9500 or the CEWAF remains to be resolved (SI Appendix).

Like *Marinobacter*, the abundance of *Cycloclasticus* increased primarily in the WAF treatments, where their relative abundance increased from 12% to 23% after 1 wk and an oligotype (type 03) closely related to *Cycloclasticus pugetii* (SI Appendix, Figs. S3B and S11), which degrades naphthalene, phenanthrene, anthracene, and toluene as sole carbon sources (33), increased substantially. *Cycloclasticus* also increased slightly in relative abundance in the CEWAF + nutrients treatment (Fig. 1B), but less so than in the WAF treatment.

*Oceanispirillum* (also known as DWH *Oceanospirillum*) (34) abundance decreased consistently across treatment, regardless of the presence or absence of WAF, dispersant, or CEWAF ( $\pm$  nutrients) (Fig. 1B and SI Appendix, Figs. S3C and S8). The observed oligotypes closely resembled those observed *in situ* during the DWH incident (18) (SI Appendix, Fig. S12). The DWH *Oceanospirillum* oxidize n-alkanes and cycloalkanes (17); cycloalkanes are absent in surrogate Macondo oil, possibly explaining the low abundance of *Oceanospirillum* in the microcosms.

**Cell Growth and Exopolymer Formation.** Initially, cell abundance was similar across treatments ( $3 \times 10^5$  cells·mL<sup>-1</sup>; Fig. 1A). At the experiment's termination, microbial abundance in WAF treatments had increased by a factor of 60, which was significantly higher ( $T_4$ :  $P < 0.0001$ ) than microbial abundance in CEWAF ( $\pm$  nutrients) treatments. Microbial abundance in dispersant-only treatments increased by a factor of 29, less than in WAF treatments but showing clear stimulation of growth by dispersant alone.

Marine oil snow, here defined as particles  $>0.5$  mm in diameter, formed in WAF, dispersant-only, and CEWAF ( $\pm$  nutrients) microcosms, but differed in appearance, size, and abundance across treatments (SI Appendix). Microbial exopolymeric substances, including transparent exopolymer particles (TEP), are a matrix for marine snow formation (35). Oil-degrading bacteria produce TEP as biosurfactants (36). TEP production increased in the WAF microcosms relative to controls, underscoring the metabolic activities of oil-degrading bacteria (SI Appendix, Table S1). The abundance of TEP could not be quantified in dispersant treatments (SI Appendix) but extensive formation of oil snow was observed in the CEWAF + nutrients treatments (SI Appendix), inferring that TEP levels were likely elevated. The macroscopic particles observed in these experiments resembled marine oil snow observed *in situ* during the DWH oil spill (SI Appendix, Fig. S13 F and G). Catalyzed reporter deposition in combination with

fluorescence *in situ* hybridization (CARD-FISH) revealed that Gammaproteobacteria and Alteromonadales, which includes the *Colwellia*, dominated microaggregate populations in CEWAF + nutrients treatments (SI Appendix, Fig. S13 P-R and SI Text). These findings suggest that *Colwellia* plays an important role in marine oil snow formation in the presence of dispersants.

**Microbial Activity and Oil and Dispersant Degradation.** Dispersant addition did not enhance bacterial oil degradation or microbial activity in general, as reflected in rates of hydrocarbon oxidation, bacterial protein production, and exoenzyme activities. Radiotracer assays allowed direct quantification of alkane ([1-<sup>14</sup>C]-hexadecane) and polycyclic aromatic hydrocarbon (PAH) ([1-<sup>14</sup>C]-naphthalene) oxidation rates across treatments (SI Appendix) (Fig. 3 A and B and SI Appendix). Hexadecane oxidation rates were significantly reduced ( $T_3$  and  $T_4$ :  $P = 0.004$ ) in dispersant-only and CEWAF ( $\pm$  nutrients) treatments (Fig. 3A), implying that dispersants suppressed hexadecane degradation. Similarly, naphthalene oxidation rates in the WAF treatments were higher than those in dispersant-only and CEWAF ( $\pm$  nutrients) treatments ( $T_3$  and  $T_4$ :  $P < 0.0001$ ), inferring that dispersants did not stimulate microbial naphthalene degradation (Fig. 3B). When substrate turnover constants instead of concentration-dependent rates were considered, inhibition of hexadecane turnover remained apparent, whereas naphthalene turnover was comparable between WAF and CEWAF treatments (SI Appendix, Fig. S14). Together, these data show a clear concentration-independent inhibition of hexadecane oxidation by dispersants and further show that dispersants did not stimulate naphthalene biodegradation rates.

To validate the patterns of rates in these deepwater samples in another Gulf habitat, we determined hydrocarbon turnover of hexadecane and naphthalene in highly oil-contaminated (SI Appendix) surface seawater samples with and without dispersant addition (dispersant to seawater dilution was 1:100,000 vol/vol). Application of the radiotracer assay demonstrated that hexadecane turnover was inhibited significantly by dispersant amendments and that naphthalene turnover was not stimulated (SI Appendix, Fig. S15). These findings mirror those observed in the deepwater microcosms and underscore their broad relevance.

Further, in the deepwater experiments, not only were rates of hydrocarbon oxidation highest in the WAF treatments, rates of bacterial protein synthesis and exoenzyme activities indicative of potential bacterial degradation rates of carbohydrate- and protein-rich exopolysaccharides (EPSs) were also maximal in WAF treatments (Fig. 3C and SI Appendix, Table S1). All enzyme assays exhibited up to one order of magnitude higher activities in the WAF and dispersant-only treatments compared with the CEWAF ( $\pm$  nutrients) treatments (Fig. 3 D-F and SI Appendix, Table S1), underscoring that dispersant-only and CEWAF ( $\pm$  nutrients) did not stimulate bacterial production ( $T_3$  and  $T_4$ :  $P < 0.001$ ) relative to the WAF treatments.

Results from gas chromatography-mass spectrometry (GC-MS) and excitation/emission matrix spectra (EEMS) in deepwater samples further confirmed the patterns of hydrocarbon degradation across deepwater treatments. Concentrations of n-alkanes and hexadecane decreased more significantly in WAF treatments (SI Appendix, Fig. S16). In the WAF treatment, microorganisms preferentially degraded low molecular weight n-alkanes ( $<C_{20}$ ) relative to high molecular weight ( $\geq C_{21}$ ) compounds and the isoprenoids, pristane and phytane. In the dispersant treatments, this pattern was not observed (SI Appendix, Fig. S17). The temporal changes in n-alkane concentration (SI Appendix, Fig. S18) supported the rate data (SI Appendix, Table S1) and emphasized the fact that oil degradation was highest in WAF treatments and that addition of CEWAF, even in the presence of additional nutrients, did not generate higher overall hydrocarbon degradation rates.

Biodegradation of anionic surfactant DOSS to  $\alpha/\beta$ -ethyhexyl-sulfosuccinate (EHSS) occurs under aerobic conditions (37). In the dispersant-only treatment, a significant ( $P < 0.05$ ) decrease (8%) of DOSS and an increase of EHSS (15%) was observed at  $T_3$  (SI Appendix, Fig. S18 A and B). The nonionic surfactants were





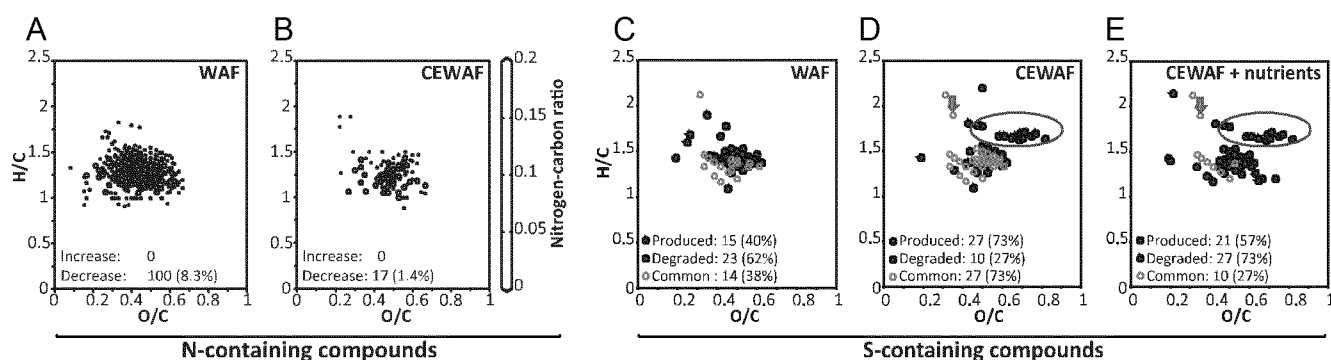


Fig. 4. Dispersants impact microbial turnover of dissolved organic matter. Analysis of molecular-level patterns in Van Krevelen diagrams (hydrogen-to-carbon, H/C, and oxygen-to-carbon, O/C ratios; each circle represents a molecular formula). (A and B) Van Krevelen diagrams showing nitrogen-containing formulae (color scale depicts N/C ratios; open circles, formula contained no nitrogen). (C–E) Van Krevelen diagrams presenting changes in the presence or absence of sulfur-containing compounds (red circles, produced compounds, i.e., absent at  $T_0$  but present at  $T_4$ ; blue circles, degraded compounds, i.e., absent at  $T_4$  but present at  $T_0$ ; open circles, common compounds present at  $T_0$  and  $T_4$ ). DOSS (molecular formula  $C_{20}H_{36}O_7S$ , marked by arrow) was present at  $T_0$  and  $T_4$ . Several sulfur-containing compounds were exclusively produced in the dispersant-amended treatments (molecular formulae marked by an ellipse).

Marinobacter oligotypes correlated positively to total petroleum concentrations (83%) and hexadecane oxidation (71%), highlighting a key role for these microorganisms in hexadecane degradation in the absence of dispersants. Oceaniserpentilla and Cycloclasticus oligotypes (30 and 31 types, respectively) correlated positively with nitrate and total n-alkanes, hexadecane, naphthalene, and phenanthrene (71–80%) concentrations. In addition, Cycloclasticus abundance positively correlated with naphthalene oxidation (61%), supporting their involvement in PAH degradation.

**Evaluating the Utility of Dispersants.** Dispersants are used regularly as a response action after oil spills to disperse oil slicks, enhance the relative oil surface area in water, and to stimulate microbial hydrocarbon degradation. During the DWH incident, the deep-sea application of dispersants was unprecedented. Prior studies about microbial dispersant impacts generated confounding results (for review see ref. 25) most likely because nonspecific metrics were used, e.g., microbial cell counts or the production of  $CO_2$ . Though changes in these two metrics reflect changes in microbial growth or activity, they do not specifically signify changes in hydrocarbon degradation rates. Further, it is quite possible that microorganisms stimulated by dispersant addition may outcompete natural hydrocarbon degraders. Thus, a direct quantification of hydrocarbon oxidation, accomplished here by direct determination of hydrocarbon oxidation using radiotracer assays in tandem with hydrocarbon quantification by GC-MS, is necessary to elucidate the impacts of dispersants on microbial populations and activities. The data obtained do not support dispersant stimulation of oil biodegradation, questioning the utility of dispersant application to pelagic ocean ecosystems.

Dispersant impacts on pelagic environments that are not impacted by natural oil seepage remain largely unknown. However, it seems unlikely that dispersants would stimulate hydrocarbon degradation in a system that lacks a substantial population of hydrocarbon degraders when they had no stimulatory effect in samples from a system that was primed for oil degradation (e.g., oil degraders account for 7–10% of the natural microbial population at site GC600) (18). In fact, the presence of dispersant selected against the most effective hydrocarbon degrading microorganisms (i.e., Marinobacter). This multidisciplinary data set strongly suggests that dispersants did not stimulate microbial hydrocarbon-degradation rates, as maximal oil-degradation rates were observed in the WAF treatments. Though we quantified degradation rates of only two hydrocarbons, hexadecane and naphthalene, biodegradation of other n-alkanes and PAHs could be similarly affected by dispersants. Quantification of the total crude oil also showed that the highest levels of oil biodegradation occurred in treatments without dispersants.

Whereas microbial activities in CEWAF ( $\pm$  nutrients) microcosms were comparable for 1 wk, rates were stimulated by nutrients in the later time points (e.g., CEWAF + nutrient hydrocarbon oxidation rates after 4 and 6 wk), suggesting progressive nutrient limitation. Clearly, the Gulf's deepwater microbial community is able to degrade oil efficiently in the absence of dispersant. Therefore, caution is advised when considering dispersant applications as a primary response for future oil spills in deepwater environments similar to the Gulf. A full understanding of dispersant impacts on microbial populations requires immediate and careful evaluation of dispersant impacts across a variety of habitats.

## Materials and Methods

**Microcosm Setup and Sampling.** Seawater (160 L) was sampled from 1,178 m at an active natural hydrocarbon seep in the northern Gulf on March 7, 2013 (site GC600, latitude 27.3614, longitude -90.6018; SI Appendix, Fig. S1). After sampling, seawater was transferred to 20 L carboys and stored at 4 °C onboard the ship for 3 d. The carboys were transported at 4 °C to the laboratory at University of Georgia where the experiment and sampling were conducted in an 8 °C cold room. Setup and sampling of microcosms are described in detail in SI Appendix, SI Materials and Methods. In brief, 72 2-L glass bottles (1.8-L sample per bottle) were incubated on a roller table (SI Appendix, Fig. S2). Treatments (WAF, dispersant-only, and CEWAF  $\pm$  nutrients) and controls (abiotic and biotic) were run in triplicate for each time point. Sampling (except for the CEWAF + nutrients treatment) was performed after 0 d ( $T_0$ ), 1 wk ( $T_1$ ), 2.5 wk (16 d;  $T_2$ ), 4 wk ( $T_3$ ), and 6 wk ( $T_4$ ); CEWAF + nutrients treatments were sampled at  $T_0$ ,  $T_1$ , and  $T_4$ . CEWAFs were prepared by mixing pasteurized seawater with oil and/or dispersants for 48 h at room temperature and subsequently subsampling CEWAFs, excluding contamination by oil or dispersants phases (SI Appendix). In addition, hydrocarbon turnover was determined in oil-contaminated surface seawater samples obtained along a transect from the Taylor Energy oil platform to the Mississippi River plume. Oil-contaminated surface seawater samples were used directly (untreated samples) or amended with dispersants (SI Appendix). Hydrocarbon turnover was analyzed using the newly adapted radiotracer assays (SI Appendix).

**Molecular, Microbiological, and Geochemical Analyses.** Nutrients (nitrate, nitrite, phosphate, and ammonium), dissolved inorganic carbon, and oxygen as well as hydrocarbons (44) and dispersant concentrations were monitored during the course of the experiment (SI Appendix). Microbial community evolution and cell numbers were investigated for each sample using 16S rRNA amplicon Illumina sequencing (Bioproject accession PRJNA253405), computational oligotyping analysis (28), and total cell counts (SI Appendix). Activity measurements were performed using enzyme assays (peptidase, glucosidase, lipase) (45),  $^3H$ -leucine incorporation analysis (46), as well as the newly developed method for the analysis of  $^{14}C$ -hexadecane and  $^{14}C$ -naphthalene oxidation (SI Appendix). TEP analyses were carried out for controls and oil-only treatments (47) and CARD-FISH analysis (48) were performed in particular for microbial-aggregate formations in nutrient treatments (SI Appendix). Oil-derived hydrocarbons were extracted from water samples using a mixture of

hexane:dichloromethane (1:1, vol/vol). After concentration, hydrocarbon compounds were identified and quantified by GC/MSD using conditions described previously (49) (SI Appendix). Analysis of the surfactant components of the dispersant Corexit was performed by LC-MS/MS as described elsewhere (13), with minor modification (SI Appendix). FT-ICR-MS was carried out to analyze DOM (50) (SI Appendix). Statistical analyses were used to unravel factors that drive microbial community evolution and microbial activities (SI Appendix).

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used in the experiments; Julie Huber and Wade Jeffrey for sharing protocols for DNA extraction and WAF preparation, respectively; Kim Hunter for conducting nutrient and DOC analyses; Vladimir Samarkin for assistance during radiotracer assay development; and the Microbial Diversity Course (coordinated by Steven Zinder and Daniel H. Buckley) at the Marine Biological Laboratory, for providing supplies for CARD-FISH and access to the laser-scanning fluorescence microscope. This research was supported by a grant from British Petroleum/the Gulf of Mexico Research Initiative to support the "Ecosystem Impacts of Oil and Gas Inputs to the Gulf (ECOGIG)" consortium. P.M.M. also acknowledges funding from the National Science Foundation (OCE-1057683). This is ECOGIG contribution no. 347 and the data are archived at Gulf of Mexico Research Initiative Information and Data Cooperative data set number R1.x132.135.0012.

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**From:** Mandsager, Kathy  
**Sent:** Fri 2/27/2015 8:44:47 PM  
**Subject:** Dispersant Science in Arctic Waters - Degradation and Fate  
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Dear Degradation & Fate group members:

Our next meeting to discuss the outstanding items on this document, particularly with information from the older published papers (LUMCON) that address biodegradation, will be held **Wednesday, March 11 beginning at 1:30 pm ET**. Please mark your calendar and plan to participate.

Attached is the biodegradation spreadsheet for this discussion.

This meeting will be via WebEx and the instructions are noted below.

### **Degradation & Fate Group**

Wednesday, March 11, 2015

1:30 pm | Eastern Daylight Time (New York, GMT-04:00) | 3 hrs

### **Join WebEx meeting**

Meeting number: 312 666 165

### **Join by phone**

**1-855-244-8681** Call-in toll-free number (US/Canada)

**1-650-479-3207** Call-in toll number (US/Canada)

Access code: 312 666 165

[Global call-in numbers](#) | [Toll-free calling restrictions](#)

Can't join the meeting? [Contact support](#).

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# Biodegradation Database

#	Title	Author
1	A new approach in enhanced biodegradation of spilled oil: Development of an oil dispersant containing oleophilic nutrients	R.D.E. Bronchart, J. Cadron, A. Charlier, A.A.R. Gillot, W. Verstraete
2	Asphaltene biodegradation using microorganisms isolated from oil samples	Tavassoli, T., S. M. Mousavi, S. A. Shojaosadati, and H. Salehizadeh
3	Behavior of a chemically dispersed oil in a wetland environment	C.A. Page, J.S. Bonner, T.J. McDonald, R.L. Autenrieth
4	Behavior of chemically dispersed oil in a wetland environment	C.A. Page, R.L. Autenrieth, J.S. Bonner, T.J. McDonald
5	Biodegradability of Corexit 9500 and Dispersed South Louisiana Crude Oil at 5 and 25 °C	P. Campo, A. D. Venosa, M. T. Suidan
6	Biodegradability of dispersed crude oil at two different temperatures	A.D. Venosa and E.L. Holder

**7** Biodegradation of Dispersed Forties Crude and Alaskan  
North Slope Oils in Microcosms Under Simulated Marine  
Conditions S.J. Macnaughton, R. Swannell, F.  
Daniel, L. Bristow

**8** Biodegradation of Dispersed Oil in Arctic Seawater at -1°C K. M. McFarlin, R. C. Prince, R.  
Perkins, M. B. Leigh

**9** Biodegradation of petroleum hydrocarbons at low  
temperature in the presence of the dispersant Corexit 9500 J.E. Lindstrom & J.F. Braddock

**11** Biodegradability of dispersed crude oil and two different temperatures

A.D. Venosa, E. L. Holder

**12** Comparative Fate of Chemically Dispersed and Beached Crude Oil in Subtidal Sediments of the Arctic Nearshore

P.D. Boehm, M.S. Steinhauer, D.R. Green, B. Fowler, B. Humphrey, D.L. Fiest, and W.J. Cretney

**13** Deep-sea bacteria enriched by oil and dispersant from the DWH spill

J. Baelum, S. Borglin, R. Chakraborty, J. L. Fortney, Regina Lamendella, O. U. Mason, M. Auer, M. Zemla, M. Bill, M. E. Conrad, S. A. Malfatti, S. G. Tringe, H-Y. Holman, T. C. Hazen, J. K. Jansson

**14** Dispersion and biodegradation of oil spills on water

R. Varadarak, M.L. Robbins, J. Bock, S. Pace, D. MacDonald

**15** Effect of Biological and Chemical Dispersants on Oil Spills R.I. Abdallah, S.Z. Mohamed, and F.M. Ahmed

**16** Effect of Dispersants on Oil Biodegradation Under Simulated Marine Conditions R.P.J. Swannell & F. Daniel

**17** Effect of Initial Oil Concentration and Dispersant on Crude Oil Biodegradation in Contaminated Seawater Mohammad Ali Zahed, Hamidi Abdul Aziz, Mohamed Hasnain Isa, Leila Mohajeri

**18** Effect of Salinity on Biodegradation of Oil Spill Dispersants G.C. Okpokwasili & L.O. Odokuma

**19** Effects of chemical additives on hydrocarbon disappearance and biodegradation in freshwater marsh microcosms J.A. Nyman, P.L. Klerks, S. Bhattacharyya

- 20** Effects of Dispersant Addition on Diesel Oil Biodegradation in Marine Environment Z. Xilai, W. Guizhi, L. Shuqing, Li. Jincheng
- 21** Effects of nutrient and temperature on degradation of petroleum hydrocarbons in sub-Antarctic coastal seawater Delille D, Pelletier E, Rodriguez-Blanco A, Ghiglione GF
- 22** Effects of Short-Term Exposure to Dispersed Oil in Arctic Invertebrates C. Mageau, F.R. Englehardt, E.S. Gilfillan, and P.D. Boehm
- 23** Effects of three types of oil dispersants on biodegradation of dispersed crude oil in water surrounding two Persian Gulf provinces A. ,Zolfaghari-Baghbaderani,Emtyazjoo, M., Poursafa, P., Mehrabian, S., Bijani, S., Farkhani, D., Mirmoghtadaee, P.
- 24** Evaluating the biodegradability and effects of dispersed oil using arctic test species and conditions: Phase 2 activities K.M.,McFarlin ,Perkins, R.A., Gardiner, W.W., Word, J.D.
- 25** Evaluation of biocompatibility and biodegradation of three different oil dispersants in Persian Gulf: Siri Island water B.A.,Zou Alfaghari,Mehrabian, S., Emtiazjoo, M., Farkhani, D., Hosseini, S.M.
- 26** Evaluation of bioremediation effectiveness on crude oil-contaminated sand San-Jin Kim, Dong Hyuk Choi, Doo Suep Sim, Young-Sook Oh
- 27** Fate and Effects of Dispersed Crude Oil Under Icy Conditions Simulated in Mesocosms R. Siron, E. Pelletier, D. Delille, and S. Roy
- 28** FINASOL OSR 52 Active Components Biodegradation by Using the Biological Activator BIOLEN IG 30 J.R. Bergueiro-Lopez, S. Moreno-Garcia-Luengo, F. serra-Socias, A. Fuertes-Perez, A. Perez-Navarro-Gomez, N. Morales-Correas, and F. Dominguez-Laseca

- 29** Kinetic modeling and half life study on bioremediation of crude oil dispersed by Corexit 9500 M.A.,Zahed,Aziz, H.A., Isa, M.H., Mohajeri, L., Mohajeri, S., Kutty, S.R.M.
- 30** Level and Degradation of Deepwater Horizon Spilled Oil in Coastal Marsh Sediments and Pore-Water M. Nutter, J. Keevan, Y. Wang, A. R. Keimowitz, B. C. Okeke, A. Son, M-K. Lee
- 31** Microbial degradation of resins fractionated from Arabian light crude oil Venkateswaran, Kasthuri, Toshihiro Hoaki, Misako Kato, and Tadashi Maruyama



**32** Microbial Response to Crude Oil and Corexit 9527:  
SEAFLUXES Enclosure Study

K. Lee, C.S. Wong, W.J. Cretney,  
F.A. Whitney, T.R. Parsons, C.M.  
Lalli, and J. Wu

**33** Naphthalene biodegradation in temperate and arctic  
marine microcosms

Bagi A, Pampanin DM, Lanzén A,  
Bilstad T, Kommedal R

**34** Natural and Stimulated Biodegradation of Petroleum in  
Cold Marine Environments - BOOK

O. G. Brakstad

**35** Nutrient Effects on the Biodegradation Rates of Chemically-  
Dispersed Crude Oil

B.C. Harris, J.S. Bonner, T.J.  
McDonald, C.B. Fuller, C.A. Page,  
P. Dimitriou-Christidis, M.C.  
Sterling, R.L. Autenrieth

**36** Optimisation of oil spill dispersant composition by mixture design and response surface methods J. Brandvik & S. Daling

**37** Rapid degradation of Deepwater Horizon spilled oil by indigenous microbial communities in Louisiana saltmarsh sediments N. Mahmoudi, T. M. Porter, A. R. Zimmerman, R. R. Fulthorpe, G. N. Kasozi, B. R. Silliman, G. F. Slater

**38** Study on the fate of petroleum-derived polycyclic aromatic hydrocarbons (PAHs) and the effect of chemical dispersant using an enclosed ecosystem, mesocosm M. Yamada, H. Takada, K. Toyda, A. Yoshida, A. Shibata, H. Nomura, M. Wada, M. Nishimura, K. Okamoto, K. Ohwada

**39** The Biodegradation Characteristics of the Mixtures of Bunker-A, B Oils with Dispersants in the Seawater Joong-Soo Baek, Gwang-Su Kim, and Eun-il Cho

**40**

The primary biodegradation of dispersed crude oil in the  
sea

Roger C. Prince, Kelly M.  
McFarlin, Josh D. Butlera, Eric J.  
Febboa, Frank C.Y. Wang, Tim J.  
Nedwedd

Abbreviations: NOS - not otherwise specified

## Oil

Year	Oil Source	Oil Type	Oil Concentration
1985	Kuwait crude	Kuwait crude 150	1 g of nutrient, 7 g of dispersant, 28 g of Kuwait 150 and 0.7 liter of sea water all mixed together
2012	Asphaltene medium extracted from crude oil	NOS	10 g asphaltene
2002	Texaco/Texas Gulf Coast	Arabian medium crude oil	21L of oil was mixed with 2.1 or 1/05 of dispersant
2001	Texaco/Texas Gulf Coast	Arabian medium crude oil	21L of oil was mixed with 2.1 or 1/05 of dispersant
2013	South Louisiana crude	Reference crude (similar to Macondo)	
2007		Fresh Prudhoe Bay crude oil (PBC)	

2003 Forties crude,  
Alaskan North  
slope (ANS)

Alaska north slope  
2014 crude Collected from  
Alyeska terminal  
in 2009, some  
artificially  
weathered 2.5 ppm, 15 ppm

2002 fresh or  
weathered ANS 90 mg fresh or 90 mg 1:10 dispersed fresh  
crude oil ANS

Prudhoe Bay crude 2007 oil	Fresh PBC	100 µL
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2015	Lagomedio crude oil (artificially weathered)
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2012 MC252

1995	521-1050 F distillation fraction of Alaska North Slope crude (ANS 1050)
------	--

2003 NOS

NOS

NOS

1999

weathered  
Forties crude oil  
(4ml)

Shell Refining  
Company, Port  
2010 Dickson, Malaysia

Light crude oil 100, 500, 1000, 2000 mg/L

1990

2006

South Louisiana  
crude oil and  
diesel

2009	Diesel oil 0#	Diesel from Shengli oilfield	0.2 mL
2009		Crude oil, diesel	5, 0.5 mL
1987		BIOS-stock aged Lagomedio crude oil	
2011		Crude oil	
2009			
	western coast of Korea and sieved 2004 prior to use	Arabian light crude	
1993	North Sea	Forties	124 ppm
1997			



Crude oil was a mixture of Tapis, Bintulu, Miri Light and Sutu den with percentages of 54, 17, 5 and 24%, 2011respectively.	Crude mix	100, 500, 1000, 2000 mg/L
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2012Macondo crude	Deepwater Horizon Macondo
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2011Arabian light crude	Light crude oil	5000 ppm
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1985	Prudhoe Bay crude oil	0.3ppm and 3.0ppm
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2014 Naphthalene	Six contained naphthalene (10 mg L <sup>-1</sup> ) as the sole carbon source, two received sodium-benzoate (10 mg L <sup>-1</sup> ) as positive controls, one had no additional carbon (blank) and one was prepared with naphthalene (10 mg L <sup>-1</sup> ) and sodium-azide (1 g L <sup>-1</sup> ) to diminish bacterial activity (negative control). Flasks containing naphthalene were prepared as follows: seawater (800 mL) was transferred into a 1 L flask and 200 mL naphthalene stock solution (50 mg L <sup>-1</sup> prepared in heated distilled water) together with sea salts was added to adjust salinity to 35 psu
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2008		
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2002	weathered Arabian medium crude	
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1998 North Sea crudes	Statfjord and Oseberg (together with medium bunker fuel (IF-3)) - artificially weathered for 6 hours at sea
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2013 Macondo crude	MC 252
--------------------	--------

2003

1996	Bunker-A, Bunker B
------	-----------------------

Alaska north slope  
2013 crude

2.5 ppm

## Water

Source of seawater	Type of seawater	Volume	Microbial Community
	Synthetic		Bacteria grown on Kuwait crude oil and originally came from sewage treatment plant. Culture contains 59 mg/l Nitrogen
Soil samples from Dorood oilfield			Pseudomonas, Bacillus licheniformis, Bacillus lentus, Bacillus cereus, Bacillus firmus. Isolated from Dorood oilfield in Southern Iran
	Due to low salinity water, a sumplement of a divalent ion salt that improved the dispersant's performance in fresh water was added		
	Due to low salinity water, a sumplement of a divalent ion salt that improved the dispersant's performance in fresh water was added		
Meso (surface) and cryo (close to well)	Gulf of Mexico seawater	100 mL	Indigenous seawater bacterium
Artificial seawater (GP2)	3.5% artificial salt water		Isolated from Disk Island, near EVOS. An undefined mixed consortium grown on PBC fro 3 weeks in a 10L batch

(1) seawater with oil and Corexit 9500, (2) seawater with oil, Corexit 9500, and added nutrients (1 mg N-NO<sub>3</sub>/l), and (3) seawater with oil, Corexit 9500, and 300 mg/l mercuric chloride

Mesocosm containing Arctic seawater collected from Chukchi Sea, Alaska

Free of slush and ice, 1 km from Barrow, 1 m below ice

artificial seawater (SW; Crystal Sea® Marine Mix, Marine Enterprises International, Baltimore, MD)

From frozen (-80C) stock source. Stocks created from secondary batch enrichment cultures using 500 ml sterile marine mineral nutrient broth inoculated with 1 ml primary batch enrichment culture and amended with ~5 mg phenanthrene and ~180 mg ANS crude oil per 1

Artificial seawater, GP2	Artificial, salinity 3.5%	120 mL	Disk Island isolation, undefined mixed consortium grown on PBC
		15m3	
Gulf of Mexico			Inoculum in uncontaminated seawater collected during spill at 1100mbsl
			source was sludge from a refinery biological oxidation unit

near Eddystone Lighthouse, UK		15L natural seawater per microcosm	
natural			
Penang Island, Malaysia	Malaysian seawater from the Penang Island area, Perai area	250 mL	Malaysia Isolates seawater
Raw riverwater: New Calabar River, 200m west of University of Port Harcourt			
Freshwater	freshwater in laboratory microcosms in freshwater marshes		



Natural source (36° 03'N, 120° 20'E)	100 mL	Mixed inoculum from oil-polluted seawater from Dagang wharf No. 6
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Coastal seawater of Kerguelen Archipelago (49°22'S, 70°12'E)	Surface coastal seawater	600 mL
--	--------------------------	--------

offshore intake near Cape Hatt on northern Baffin Island	natural	
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Persian Gulf, Siri and Bahregan provinces		Indigenous seawater bacterium
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Siri Island Water

sterilized aged seawater	Each of the three oil-degraders
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3m depth from St. Lawrence Estuary in Quebec, Canada	natural	
--	---------	--

Peraí area, Butterworth,  
Malaysia

Malaysian seawater

Acinetobacter,  
Alcaligenes, Bacillus,  
Pseudomonas and Vibrio.

Three heavily contaminated  
zones in Louisiana (Bay Jimmy,  
Bay Batiste, Bayou Dulac) and  
three intermediate sites in  
Alabama (Walker Island),  
Mississippi (Point Aux Chenes  
Bay), and Louisiana (Rigolets),  
as well as three unaffected sites  
in Alabama (Weeks Bay, Longs  
Bayou) and Mississippi (Bayou  
Heron)

Gulf of Mexico seawater

Indigenous

Sediment from Japanese coast

A. Pseudomonas sp

Obtained adjacent to  
enclosures

Byfjorden, Norway	80 m depth, collected via pipeline filterd through 10 µm	1 L	Indigenous
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Corpus Christi Bay

Salt marsh sediments in Gulf of Barataria Bay, Louisiana. Two  
Mexico, 3 m from marsh impacted sites and two reference  
platform edge site samples

*Spartina alterniflora* -  
dominated

Hamana Bay natural, but with high salinity  
(30.8-32.4psu)

natural

New Jersey shore seawater in  
April 2010 and 2011

NJ - salinity 28 ppt, temp 8 C

4 L

Indigenous microbiota

## Methods

Nutrients

Temperature

Analytical Constituent s

27.5 g/l NaCl, 7.0 g/l MgSO<sub>4</sub>\*H<sub>2</sub>O, 5.2 g/l  
MgCl<sub>2</sub>\*6H<sub>2</sub>O, 0.7 g/l KCl, 0.01 M K<sub>2</sub>HPO<sub>4</sub>,  
0.26 g CaCl<sub>2</sub> \* 2 H<sub>2</sub>O

150 C

Chromatographic analysis

pH of 7.4

28 C

Asphaltene

Indigenous

5, 25 C

DOSS, alkanes, aromatics

5 C and 20 C

For ANS, on day 8, 1 mg/l sodium nitrate 15 C (Forties), 8  
and 0.1 mg/l potassium dihydrogen C (Alaskan North  
orthophosphate was added slope)

pH (8.05),  
temperature (21uC), dissolved oxygen  
(11.6 mg/L) and salinity  
(33 ppt). Nutrient levels (nitrate, nitrite,  
and ammonia) were below  
detection limits by simple colorimetric  
tests

-1 Petroleum hydrocarbons

see Microbial Community

8C

5, 20 C

Hydrocarbons

Indigenous, Fe added

5 C

Hydrocarbons, microbial  
community



Low levels of N and P, High levels of N and  
P, low levels of nutrients + mercuric  
chloride 15C

C:P:N 100:10:1 28 C CO, DCO, TPH  
The mineral salts broth had in L:  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.4 g/L; KCl, 0.28 g/L;  $\text{KH}_2\text{PO}_4$ , 0.8 g/L;  $\text{K}_2\text{HPO}_4$ , 1.2 g/L;  $\text{NaNO}_3$ , 0.4 g/L, and pH 7.4. ambient

Salinities of 11, 22 and 33 g/L                      10, 15, and 20 C   TPH

CNP = 62:7.4:0.7 (added fertilizer Inipol)   4, 10, 20 C                      Total alkanes

30 C                      BOD, COD

C:N:P ratio of 100:10:3 from slow release  
inorganic fertilizer                      25C

20C

CNP = 100:10:1

NOS

TPH

DOC

phosphate:nitrate:silicate /  
1:10:10micrometers  
2.8Mum P04, 25Mum MNO2 and NO3,  
and 50Mum MSiO4.

Inorganic nutrients were added (16.2 mg L<sup>-1</sup> K<sub>2</sub>HPO<sub>4</sub>, 0.8 mg L<sup>-1</sup> KH<sub>2</sub>PO<sub>4</sub>, 42.0 mg L<sup>-1</sup> NaNO<sub>3</sub>, 0.05 mg L<sup>-1</sup> FeCl<sub>3</sub>, 2.5 mg L<sup>-1</sup> CaCl<sub>2</sub> and 1.5 mg L<sup>-1</sup> MgSO<sub>4</sub>). Trace minerals were added according to Balch et al. (1979). Amino acids (10 lL L<sup>-1</sup> RPMI 1640 amino acids solution 509, Sigma) and vitamins (10 lL L<sup>-1</sup> of a stock solution with 20 mg L<sup>-1</sup> myoinositol, 0.1 mg L<sup>-1</sup> thiamine-hydrochloride, 0.1 mg L<sup>-1</sup> pyridoxine-hydrochloride, 1.0 mg L<sup>-1</sup> nicotinic acid, 0.5 mg L<sup>-1</sup> glycine, 0.01 mg L<sup>-1</sup> biotin and 0.1 mg L<sup>-1</sup> folic acid) (modified from Balch et al. 1979) were also added.

0.5, 4, 8, 15 C      Naphthalene

Nitrogen and Phosphorus added

Indigenous salt marsh nutrients in Gulf of Mexico

Alkanes and polycyclic aromatic hydrocarbons

Fertilizer (NO<sub>3</sub>-100 lg-N/L, PO<sub>4</sub>-10 lg-P/L) was added to all tanks prior to the experiment to promote primary production

20C

Total oxygen demand analyzer, element analysis, estimation of biodegradability

Indigenous - Nitrate and phosphate levels  
below detection with colorimetric test but  
likely near 7 and 0.5  $\mu\text{M}$  8 C

3-ring aromatics, 2-ring  
aromatic + 1-saturated ring  
or 5-saturated rings, 2-ring  
aromatics or 4-saturated  
rings, 1-ring aromatic + 1-  
saturated ring or 3-  
saturated rings, 1-ring  
aromatic or 2-saturated  
rings, 1-saturated ring,  
alkanes, and hopanes.

## Methods

Analytical Methods

Methods and materials

DOR

Fourier transformed infrared spectroscopy  
(Nexus-670, Thermo Nicolet Co)

was carried out at 28 C and 200 rpm for 60 days. The screening was followed by transferring serial dilutions of samples from this culture onto the nutrient agar plates. The plates were then incubated at 30 C for 24 h. The biodegrading ability of isolates was evaluated using IP143 method. The asphaltene was precipitated in n-heptane using a proportion of 20:1 (n-heptane/asphaltene). There were kept in magnetic bar agitation for 18 h. Then, it was filtrated by vacuum using Whatman No. 42 filter. The precipitated

1:10 and 1:20

1:10 and 1:20

LC/MS/MS for DOSS, GC/MS for alkanes  
and aromatics

Chemicals and Reagents  
EPA provided the South Louisiana

ANOVA

1:25

involved, oil and dispersant were premixed before addition to the experimental chambers. The biodegradation of dispersed oil was tested at 1:20 and 1:15 dispersant to oil ratios (DORs) and the mineralization of Corexit 9500 alone was measured at 50 ppm. The 1:20 DOR application rate is the target ratio in oil spill response, although ratios as high as 1:10 have been required with more emulsified and viscous heavy oils [19].

#### Biodegradation experiments

Low concentrations of oil were tested (2.5 ppm and 15 ppm) in order to assess the biodegradation of dispersed oil at concentrations that are expected to approach those found in the water column after successful dispersion. Two methods were used to quantify the biodegradation of oil. The first measured the primary biodegradation of the oil, i.e. the chemical disappearance of specific hydrocarbons, monitored with respect to a conserved internal marker within the oil (hopane) [30]. Primary biodegradation was measured by adding 5 mg phenanthrene and 180 mg ANS crude oil, and incubating the culture on a shaker for 72 h. Sterilized, 40-ml septum vials (I-Chem Research, Hayward, CA) were used as microcosms. For experiments including sediment as a treatment, 1 g dry, sterile marine sediment was added to each vial. In "low oiling" experiments, 14 mg ANS was added to each vial (nominal concentration of 1400 mg/l), and in "high oiling" experiments 45 mg oil was added (nominal concentration of 4500 mg/l). Dispersant was added to oil at the rate of 1:10 (w/w) or 1:20 (w/w). After adding culture broth (10 ml each) the microcosm vials were shaken vigorously by hand for 30 s.

After the microcosms were constructed, 50 l of a 2-g/l radiolabeled hydrocarbon solution (in acetone) was added to each vial, resulting in an initial concentration of 100 lg per vial (radioactivity 100,000 dpm). Substrates used (Sigma C

GC/MS

GC/MS



GC/MS

2.1. Crude oil  
Fresh Prudhoe Bay crude oi

1:25

Synchronous ultraviolet  
spectrofluorometry, fused silica capillary  
gas chromatography with flame ionixation  
detection, and computer-assted capillary  
gas chromatographic mass spectrometry

1:10

On 20 April 2010, high-pres

Regression analysis

Gas chromatography

Chamber slide method (oil droplet analysis),  
the Most Probable Number technique (for  
microbial biomass), gas chromatography  
(oil residue analysis)

GC

Four locations were selected  
northwest Malaysia for sampling  
(1) Batu Ferringhi Beach on 20 to 1

infra red spectroscopy, BOD determination

analyzing TPHG, TPHFID, TPHMS, and TTAH  
as a three by three factorial with repeated  
measures. Statistical tests were made using  
Proc GLM of SAS Software

cleaner or dispersant  
added where appropriate  
at a volume of 1/5th of  
that of the hydrocarbon

	<p>and then the value of TPH was quantified by Oil Analyzer of infrared spectrometer (Jilin BeiGuang Optical Instrument Factory, China).</p> <p>All the chemicals used in this research are analytical grade and were purchased from certified laboratories and suppliers.</p> <p>D. Calculation method</p> <p>The value of oil degrading efficiency on day x is the average of three samples, and the difference in TPH values between</p>
Oil analyzer of infrared spectrometer	<p>1:10, 1:20, 1:30, 1:40, 1:50</p>
GC/MS	
Gas chromatography	1:10
Spectrophotometry	We selected two provinces 1 to 20
electron transport system	
estimated with the MPN method using basal mineral medium'	

GC

## 2.1. Sampling

Samples were collected from 20 to 1

A Shimadzu TOC-V Combustion Analyzer was used to measure dissolved organic carbon (DOC) in pore-water and surface water samples. Total carbon content (TOC) of bulk sediment samples (0.25–0.5 g) were analyzed following the Dumas method(31) with a LECO carbon analyzer. In order to fingerprint the sources of organic matter, sediments, marsh plants, the initial BP MC 252 oil, and weathered BP oil (scraped off oiled plants) were analyzed for carbon isotope ( $^{13}\text{C}/^{12}\text{C}$ ) ratios using a Carlo Erba Elemental Analyzer (EA) connected to a Finnigan MAT Delta Plus XP Stable Isotope Ratio Mass Spectrometer through a ConFlo III interface.

Sediment Coring and Sampling  
The sampling sites (Figure 1

Quantitative counts of potential bacterivores, observable morphological changes in bacteria using CEE-3, size distribution analysis of C-labeled particulate material

"similar to those in field application"

GC

Experimental setup  
Biodegradation of naphthalene in seawater samples was followed by measuring oxygen consumption in closed bottle system. The measuring principle has been previously described (Kjellerud et al. 2004). Two sets of experiments were run at four different incubation temperatures (15 °C, 20 °C, 25 °C, 30 °C). Incubation times were

series II gas chromatograph, autoanalyzer for ammonium, nitrate, and orthophosphate analyses, Clark-type oxygen electrode for DO, most probably number method for microbial populations

10:01

Detailed in Silliman et al. 2012 AMOP

Study Site and Sample Colle  
All sampling sites were loca

gas chromatography-mass spectrometry

1:10, 2:10, 3:10, 5:10

GC/MS

Seawater was collected from 1:15, 1:20

## Biodegradation Treatments

## Duration

## Dispersant Type

3 weeks

Finasol OSR 7

Comparing degradation of microorganism types, optimal salinity, pH and asphaltene concentration were considered

2 months

None

99 days

Corexit 9500

99 days

Corexit 9500

Corexit 9500 alone, SLC alone, SLC dispersed by 9500

50 days

Corexit 9500

Corexit 9500 or  
JD2000



Forties: 27 days,  
ANS: 35 days      Corexit 9500

Continuously mixed

60 days

Corexit 9500

35 days

Corexit 9500

The treatments consisted of triplicate flasks of PBC dispersed with Corexit 9500 for each of the sampling events, a second set of flasks containing PBC dispersed with JD2000, an uninoculated negative control containing dispersed PBC (six flasks) and either Corexit 9500 or JD2000, and another set of flasks containing non-dispersed PBC (no-dispersant control) to compare the biodegradability of dispersed and non-dispersed oil

Corexit 9500,  
28 days for 20 C arJD2000

Boehm et al. 2015 Corexit 9527

With and without dispersant looking at Fe addition

0, 5, 20 days

Corexit 9500

Corexit 9500 - blend  
of Span 80 and  
Tween 80

NOS

Corexit 9500,  
Enesperse 1583,  
Finasol OSR-51, and  
Slickgone LTSW  
(10% w/w of the oil  
weight)

21 days

Different oil concentrations with and without  
dispersant

45 days

Corexit 9500

Corexit 9527 &  
Surflow OW-1 and  
Prodesolv

15 days

Corexit 9500 (used  
as dispersant) and  
Corexit 9580 (used  
as cleaner)

186 days

	22 days	from producer?
Two different oil concentrations and three temperatures		NOS
		Corexit 9527
Three different dispersant types tested, BOD COD and microorganisms analyzed	28 days	Pars 1, Pars 2, Gamlen OD4000
		Pars 1, Pars 2, Gamlen OD4000
		Tween 80
	Multiple tests, different ranges	
	26 days	FINASOL OSR 52, biological activator = BIOLEN IG 30

Effect of oil concentrations, effect of  
dispersant

45 day, 60 days    Corexit 9500

7, 15 days

Changes in bacterial size and morphology were monitored by scanning electron microscopy. Samples retained by 0.2  $\mu$ m Nuclepore filters fixed in 2% glutaraldehyde were critical-point dried with liquid CO<sub>2</sub>, gold coated, and viewed under a JEOL model JSM-35 scanning electron microscope.

22 days

Corexit 9527

Compare naphthalene biodegradation rate, temperature response and bacterial community composition of seawater samples collected in two different geographic areas: North Sea (temperate) and Arctic Ocean

28 and 48 days

28 days

Corexit 9500

Two reference sites and two impacted sites  
were compared

18 months

Corexit 9500, 9527

9 days

20 days

Two methods of adding oil to water and oil  
with dispersant and without dispersant

60 days

Corexit 9500



## Results

Special Notes

% Loss

The optimum values of pH, salinity and asphaltene concentration for asphaltene biodegradation at 40 °C were obtained for pure cultures of *B. lentus* as 6.7, 76 g L<sup>-1</sup> and 22 g L<sup>-1</sup>, respectively, and for the mixed culture as 6.4, 76 g L<sup>-1</sup> and 12 g L<sup>-1</sup>, respectively

Biodegradations with each five strains: 43%, 42%, 46%, 40%, and 43%. 48% degradation in mixed culture

There were no statistical differences in the biodegradation rates ( $k$ ; day<sup>-1</sup>) (95% confidence). This lack of statistical difference in the biodegradation rate constants suggests that biodegradation was neither positively nor negatively impacted by CDO as compared to the undispersed oil. Biodegradation rates were also determined for all treatments; it was concluded that there were no differences when comparing each dispersed-oil treatment to the oiled control. Hopane biodegradation rates were assumed negligible.

Target compound analyses indicated no significant differences in the biodegradation rates for the three oil treatments.

At 25 C - DOSS

Two different studies in one paper. Used Mackay apparatus

Microorganisms indigenous to the Chukchi Sea were found to degrade both fresh and weathered crude oil in the presence and absence of Corexit 9500 at  $-1^{\circ}\text{C}$ , with oil losses ranging from 46–61% and up to 11% mineralization over 60 days. Weathered ANS dispersed with Corexit 9500 underwent a 57% loss in Arctic seawater after 60 days in our experiment, but experienced an 88% loss in New Jersey seawater in the same time [39]. These experiments suggest that in the Arctic, ANS crude oil degrades more slowly than oil in temperate regions, but that oil losses were still substantial even at  $-1^{\circ}\text{C}$ . There is evidence that Corexit 9500 initially stimulated oil biodegradation (Figures 1 and 2), but, as expected, its effects were minimal in longer term incubations. We conclude that the biodegradation of oil in Arctic seawater is extensive at  $-1^{\circ}\text{C}$ , and that the biodegradation of dilute, dispersed oil is not inhibited by the presence of Corexit 9500. 46-61%

Biodegradation of oil appeared to be restricted to the beached oil, with no significant degradation apparently occurring subtidally. After two years, the offshore oil residues still contained low molecular weight alkanes as well as alkylated naphthalenes

See paper

Looked at the impact of  $\text{Fe}^+$  on degradation

The physiochemical properties of the used crude oil were determined according to standard methods (Institute of Petroleum, 2000; ASTM, 2000; Universal Products Co., 1985), density (IP 190), molecular weight (ASTM D2505), kinematic viscosity (ASTM D-C/ min 445), pour point (ASTM D97 ), sulphur content (ASTM D1551), carbon residue (ASTM D524), and wax content (UOP 46-85 ). Asphaltene content was determined according to (IP 143).

Control: 31.8%, 29.2%,  
26.4%, 25.2%, For 500  
mg/L 62.5%, TPH 64%

sodium concentrations at 0, 20, and 40 g/l, microbial degradation decreased with increasing salt concentration

30-60% depending on  
DOR, 15-80%  
depending on temp,  
15-60% depending on  
salinity. Optimal 2:10  
DOR, 33ppt salinity, 30  
C

Evidence of the high potential of indigenous Antarctic bacterial communities for bioremediation action even at low temperatures. Little difference in data obtained under three incubation temperatures and with two different concentrations of oil is clearly indicating that temperature had only a rather limited influence on petroleum degradation in the studied Antarctic seawater

Pars 1 and Pars 2 were the most effective dispersants with highest degradability comparing Gamlen. In each region, the most suitable compound for removing oil spill from offshores with least secondary contamination should be investigated.

Bacteria growth

created ice cover in lab to simulate weathering processes

It has been verified that BIOLEN IG 30 biological activator is adequate for degradation of the ionic and anionic surfactants in FINASOL OSR 52 at room temperature and controlled 20°C.

Dispersant was more effective for higher oil concentration and a maximum dispersant efficiency (DE) of 38% was observed on day 15. A significant correlation between initial oil concentration and amount of TPH reduction was observed: lower initial oil concentrations exhibited higher removal efficiencies in all experiments. First order kinetics described the crude oil biodegradation with and without dispersant. Half life times of 31, 40, 50 and 75 days were observed for crude oil concentration of 100, 500, 1000 and 2000 mg/L, respectively. These were reduced, respectively, to 28, 32, 38 and 58 days with the usage of dispersant. The best hydrocarbon removal of 67% was obtained for initial crude oil concentrations of 100 mg/L. Furthermore, the most efficient removal for low initial concentrations of dispersed crude oil occurred within the first 30 days. 67% for 100mg/L oil concentrations

contents of shallow oiled sediments may have been reduced by mixing with low-DOC surface water (i.e., rainwater or seawater). At heavily oiled sites, sediment TOC levels are generally lower near the sediment surface, followed by notable increases right below. The TOC contents in uppermost sediments may be reduced by microbial degradation and the use of dispersants. Abundance of SRB and elevated sulfide concentrations in pore-waters extracted from heavily oiled Louisiana sites suggest that anaerobic sulfate reduction may be enhanced by the influx of oil and organic matter. High organic matter content and bacterially mediated sulfate reduction facilitate the formation of metal sulfides found in marsh sediments. GC-MS full-scan analysis shows significant degradation of lighter compounds, while heavier oils persist in sediments. High sensitivity GC-MS-SIM biomarker analysis clearly correlates M-252 crude oil to organic compounds extracted from marsh sediments down to 15 cm. Our carbon isotopic measurements show that the spilled BP oil has a unique  $\delta^{13}\text{C}$  signature that is significantly different from those of Louisiana salt marshes dominated by C4 plants (*Spartina* sp.). Such a large carbon isotopic difference between the marsh vegetation and the oil provides an excellent opportunity to examine the source and movement of spilled oils in coastal marshes.

A mixed population that could degrade 35% of 5000 ppm resin in 15 days was obtained. This population also metabolized 50% of saturates and aromatics present in crude oil (5000 ppm) in 7 days. A *Pseudomonas* sp. isolated from the mixed population emulsified and degraded 30% of resins. This strain also degraded saturates and aromatics (30%) present in crude oil (5000 ppm). This is the first report describing organisms that are able to grow on the resin fraction of crude oil as a sole source of carbon and energy

Biodegradation appeared to be more significant than abiotic processes in contributing to the loss of low volatility n-alkanes in Corexit-dispersed oil.

In the temperate experiment, the initial naphthalene concentration was below the designated concentration ( $10 \text{ mg L}^{-1}$ ); the mean initial concentrations were 2, 6.6, 6.2 and  $6.2 \text{ mg L}^{-1}$  at 0.5, 4, 8 and  $15^\circ \text{C}$  respectively. In the arctic experiment, the initial naphthalene concentrations were above the designated concentration (with only one exception). The mean initial concentrations were 14.4, 12.0, 14.2 and  $3.9 \text{ mg L}^{-1}$  at 0.5, 4, 8, and  $15^\circ \text{C}$ , respectively

This paper is a continuation of a previous paper in this journal entitled 'Use of statistical simulations to evaluate the advantage of designed experiments and response surface methods' - Most details presented there

Five months after the spill the impacted sites had UCM concentrations of 26,465 to 50,380 mg/kg, total alkane concentrations of 1303 to 6987 mg/kg, and PAH concentrations of 16.2 to 99.4 mg/kg (Figure 1). These concentrations were 100 times higher than those of the reference sites, which had UCM concentrations of 18 to 280 mg/kg, total alkane concentrations of 17 to 52 mg/kg, and total PAH concentrations of 1.1 to 1.5 mg/kg. Following the 5 month time point, UCM, alkane, and PAH concentrations at impacted sites rapidly decreased, and, by 11 months, concentrations had been reduced by 80–90%. By 18 months, PAH, alkane, and UCM concentrations at impacted sites were almost equivalent to those at reference sites

The more dispersants are applied to the sea for the cleanup of Bunker-A or Bunker-B oil, the more decreases the dissolved oxygen level in the seawater



more than 80% of the hydrocarbons of lightly weathered Alaska North Slope crude oil were degraded in 60 d at 8 °C in unamended New Jersey (USA) seawater when the oil was present at 2.5 ppm by volume. The apparent halftime of the biodegradation of the hydrocarbons was 13.8 d in the absence of dispersant, and 11 d in the presence of Corexit 9500 – similar to rates extrapolated from the field in the Deepwater Horizon response. See special notes

Results		Citations	
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Half-Life

Citation

Abstract

PDF

Bronchart et al. 1985

scan

Tavassoli et al. 2012

Twenty-five species w<http://www.science>

Page et al. 2002

An experiment was conducted at a wetland research faci

**ABSTRACT:** *An experiment was conducte*

Page & Autenrieth 2001

Campo et al 2013

The reported persiste  
sulfosuccinate (DOSS) <http://pubs.acs.org>

Venosa & Holder 2007

Laboratory experiments were initiated to study the biodeg

For oil spills in the open sea, operational ex

Macnaughton et al. 2003

McFarlin et al. 2014

As offshore oil and ga <http://journals.plos>

Lindstrom & Braddock 2002 Our study examined the effects of Corexit 9500 and sedim

Venosa and Holder 2007      Laboratory experimer <http://www.science>

Boehm et al. 2015      scan

Baelum et al. 2012      The Deepwater Horiz[a](http://www.ncbi.nl)

Published literature indicates that oil spill dispersio

Varadarak et al. 1995

Abdallah et al. 2003

The aim of this work is to study the effect of different type

A study was undertaken on the dispersion, mic

Swannel & Daniel 1999

Zahed et al. 2010

The effects of initial o  
Corexit 9500 dispersa <http://link.springer>

Okpokwasili & Odokuma  
1990

Biochemical oxygen demand and riverwater biodeg

Nyman et al. 2006

We determined how a cleaner and a dispersant affected h

	Jincheng et al. 2009	To evaluate the influence of oil on the degradation of hydrocarbons in sediment <a href="http://ieeexplore.ieee.org/abstract/document/5344444">http://ieeexplore.ieee.org/abstract/document/5344444</a>
	Delille et al. 2009	In an attempt to evaluate the impact of oil on the degradation of hydrocarbons in sediment <a href="http://www.researchgate.net/publication/228111111">http://www.researchgate.net/publication/228111111</a>
For hydrocarbons: Bay 9 = 8.41 days, Bay 10 = 21.0 days	Mageau et al. 1987	A series of experimental studies was carried out as part of a study on the degradation of hydrocarbons in sediment
	Zolfaghari-Baghbderani et al. 2011	To determine the most effective method for the degradation of hydrocarbons in sediment <a href="http://www.hindawi.com/2011/2011/1234567">http://www.hindawi.com/2011/2011/1234567</a>
	McFarlin et al. 2011	In the event of a marine oil spill, the degradation of hydrocarbons in sediment <a href="http://www.researchgate.net/publication/228111111">http://www.researchgate.net/publication/228111111</a>
	Zolfaghari-Baghbderani et al. 2009	The main causes of oil degradation in sediment <a href="http://en.journals.sagepub.com/doi/10.1177/0022047609344444">http://en.journals.sagepub.com/doi/10.1177/0022047609344444</a>
	Kim et al. 2004	A treatability study was conducted using sea sand spiked with oil
	Siron et al. 1993	scan
	Bergueiro-Lopez et al. 1997	This paper describes a study of the degradation of hydrocarbons in sediment

31, 40, 50, 75

Zahed et al. 2011

Hydrocarbon pollution <http://www.sciencedirect.com>

Natter et al. 2012

This research investigated <http://pubs.acs.org>

Venkateswaran et al. 2011

Sediment samples from <http://www.researchgate.net>

Lee et al. 1985

The response of marine bacteria to Corexit 9527, w

Baqi et al. 2014

Naphthalene, the smæ  
hydrocarbon (PAH), is  
crude oil, its major so<http://www.ncbi.nl>

Harris et al. 2002

Chemical dispersion of oil spills may minimize adve



Brandvik & Daling 1998

Oil spill dispersants are used to enhance the rate o

Mahmoudi et al. 2013

The Deepwater Horiz<http://pubs.acs.org>

residual methylphenanthrene was  
4% at Day-2 in OIL(high) tank on  
Run #4 (half life: 1.0 day). The  
mesocosm experiments  
demonstrated that the dominant  
fate of LMW PAHs (e.g.  
naphthalene and phenanthrene)  
was rapid biodegradation with  
their half lives of 1 day. half-lives of  
chrysene were 13 days for  
OIL(high) on Run #3, 3.5 days for  
OIL(high) on Run #4, and 57 days  
for OIL on Run #5

Yamada et al. 2003

Polycyclic Aromatic H<sup>+</sup>

Baek et al. 1996

scan

Prince et al. 2013

Dispersants are impor<http://www.science>

Column1

<http://www.sciencedirect.com/science/article/pii/S0025326X02000504>

















**To:** Conmy, Robyn[Conmy.Robyn@epa.gov]  
**Cc:** Schubauer-Berigan, Joseph[Schubauer-Berigan.Joseph@epa.gov]  
**From:** Gilliland, Alice  
**Sent:** Thur 4/9/2015 12:16:49 PM  
**Subject:** FW: BSEE Peer Review on the Testing of Four Dispersants in Simulated Arctic Conditions

.....  
>>>>>  
Robyn,

Can you please reach out to Betzy Colon next week (after you return) if you have time to be a peer reviewer on this?

Thanks,

Alice

**From:** Colon, Betzy [mailto:BColon@versar.com]  
**Sent:** Wednesday, April 08, 2015 3:32 PM  
**To:** Gilliland, Alice  
**Subject:** BSEE Peer Review on the Testing of Four Dispersants in Simulated Arctic Conditions

Hi Alice,

I tried leaving a message on your phone but not sure if I was successful. I may have hung up before the message was saved. The reason I contacted you is because we are conducting a peer review for the Bureau of Safety and Environmental Enforcement (BSEE) on dispersants and wanted to see if someone from your team might be interested in participating as a reviewer. I know Dr. Venosa used to conduct research in this area but not sure if someone from EPA has taken over this research after he retired.

I provided specific information below on the peer review. Feel free to forward to anyone on your team who may be able to participate in this peer review.

Thank you,

Betzy

**Bethzaida Colon**

Environmental Scientist

Environmental Services Group





Your primary function as a peer reviewer would be to evaluate and provide written comments on the document and answer seven charge questions.

We are identifying approximately six to seven scientific experts from which five will be selected to serve as peer reviewers. The reviewers will be senior scientists with expertise/experience in oil spill response in Arctic waters and a demonstrated understanding of the methods utilized to understand the efficacy/effectiveness of chemical dispersant use.

#### TIMELINE:

We are expecting to select reviewers within the next few weeks in preparation to begin the review in early May. Reviewers will have approximately six weeks to complete their reviews and prepare written comments, following receipt of the materials and charge questions.

#### COMPENSATION:

An honorarium is being provided for the peer review and will be discussed if you are interested and available to participate in the review.

#### NEXT STEPS:

If you are interested in participating, please provide the following information:

1. An electronic copy of your CV.
2. Complete contact information (address, phone number, email).
3. Whether you will be entering this agreement as a consultant or a subcontractor through your company (subcontract - only applicable for those people that work for companies).

Once I've received the information requested above, I will send you our conflict of interest questions for you to answer and return via e-mail, along with forms requiring your signature. Before participating, you will need to confirm that there are no conflict of interest issues, either real or perceived.

We are hoping to make selections within the next few weeks and, as a result, would appreciate a prompt response from you.

Thanks, and I look forward to hearing from you.

Betzy

**Bethzaida Colon**

Environmental Scientist

Environmental Services Group



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Fax: (703) 642-6809

Email: [bcolon@versar.com](mailto:bcolon@versar.com)

Visit us at: [www.versar.com](http://www.versar.com)

**To:** McClellan, Kim[Mcclellan.Kim@epa.gov]; Schubauer-Berigan, Joseph[Schubauer-Berigan.Joseph@epa.gov]  
**Cc:** Conmy, Robyn[Conmy.Robyn@epa.gov]  
**From:** Gilliland, Alice  
**Sent:** Tue 9/15/2015 8:59:14 PM  
**Subject:** RE: IMPORTANT - NEW STICS Entries

....  
>>>>

Bryan is listed as the author, but I assume these are Robyn's?

Yes, I can review them later this week.

**From:** McClellan, Kim  
**Sent:** Tuesday, September 15, 2015 4:57 PM  
**To:** Schubauer-Berigan, Joseph; Gilliland, Alice  
**Cc:** Conmy, Robyn  
**Subject:** IMPORTANT - NEW STICS Entries

Hi Joe and Alice,

TIM	Brian	Devi	<u>ORD-</u>	Evaluation of Sorbent and Solidifier Properties and their	Abstract	9/11/2015
Approval	Dyson	Sundaravadivelu	<u>013921</u>	Impact on Oil Removal Efficiency		4:43 PM
TIM	Brian	Mobing	<u>ORD-</u>	Biodegradability of Dispersed Heavy Fuel Oil at 5 and 25	Abstract	9/11/2015
Approval	Dyson	Zhuang	<u>013917</u>	□C		4:28 PM
TIM	Brian	Yu Zhang	<u>ORD-</u>	Biodegradation of Finasol OSR 52 and Dispersed Alaska	Abstract	9/11/2015
Approval	Dyson		<u>013915</u>	North Slope Crude Oil at 5 □C and 25 □C		3:34 PM
TIM	Brian	Ruta	<u>ORD-</u>	Biodegradability Of Diluted Bitumen Oil By Kalamazoo	Abstract	9/11/2015
Approval	Dyson	Deshpande	<u>013912</u>	River Cultures In Freshwater		2:57 PM

The abstracts are due to the GoMRI Gulf Oil Spill and Ecosystem Science Conference on Thursday. Can these abstracts be reviewed and approved, so that they can be submitted on Thursday. I will be sending the abstracts, on Wednesday (9/16/2015), after Scott Jacobs completes the Internal Technical Reviews on each abstract.

Thanks,

Kim



**To:** Conmy, Robyn[Conmy.Robyn@epa.gov]  
**Cc:** Schubauer-Berigan, Joseph[Schubauer-Berigan.Joseph@epa.gov]  
**From:** Gilliland, Alice  
**Sent:** Thur 8/11/2016 8:39:05 PM  
**Subject:** BSEE report review: E12PG00037\_Draft Final Report\_ June 2016\_clean copy\_abg.docx  
E12PG00037 Draft Final Report June 2016 clean copy abg.docx

Hi Robyn,

This is really impressive work and a very well done report. Attached are my tracked changes and comments from my STICS review. I will return it to you via STICS for revision, but I want to look through the appendices first before I return it and no longer have access.

If my technical suggestions or questions don't make sense, please let me know. I did suggest some changes to a few graphs, but I don't know if we have enough time to try those out. Once you've had a chance to address the comments that are not graph-related, we could send it to the NRMRL IO review with a note that we will be updating a few of the figures. Also, Marilyn Dapper could help with some of my editorial comments if you'd like the help, especially about an acronym list and not defining acronyms multiple times within a section (and possibly from section to section).

Thanks and great work!

Alice

**To:** Conmy, Robyn[Conmy.Robyn@epa.gov]; Yeardley, Roger[Yeardley.Roger@epa.gov]; Kremer, Fran[Kremer.Fran@epa.gov]; Smith, Kelly[Smith.Kelly@epa.gov]; Chu, Karen[Chu.Karen@epa.gov]; Ferster, Aaron[Ferster.Aaron@epa.gov]; Hahn, Intaek[hahn.intaek@epa.gov]; Mazur, Sarah[Mazur.Sarah@epa.gov]; Schappelle, Seema[Schappelle.Seema@epa.gov]; Sykes, Kathy[Sykes.Kathy@epa.gov]; Slimak, Michael[Slimak.Michael@epa.gov]; Geller, Andrew[Geller.Andrew@epa.gov]; McCullough, Melissa[Mccullough.Melissa@epa.gov]; Hubbard, Carolyn[Hubbard.Carolyn@epa.gov]; Summers, Kevin[Summers.Kevin@epa.gov]; Sjogren, Mya[Sjogren.Mya@epa.gov]  
**From:** ORD\_STICS@epa.gov  
**Sent:** Thur 2/11/2016 3:54:49 PM  
**Subject:** STICS: Clearance Completion: #ORD-013915: Biodegradation of Finasol OSR 52 and Dispersed Alaska North Slope Crude Oil at 5 °C and 25 °C

The clearance for this Sustainable and Healthy Communities product is complete:

- **Product type, subtype:** Presentations and Technical Summaries, Poster
- **Product title:** Biodegradation of Finasol OSR 52 and Dispersed Alaska North Slope Crude Oil at 5 °C and 25 °C
- **Author(s):** Zhang, Y.P. Campo, R. Deshpande, M. Zhuang and R. Conmy
- **Initiator:** Robyn Conmy, ord/nrmrl/lrpd/esmb
- **ORD Tracking Number:** Tracking # ORD-013915
- **Product Description / Abstract:** A study was conducted with Finasol OSR 52 dispersant and Alaska North Slope (ANS) crude oil in sterile GP2 artificial seawater to investigate the biodegradability of the dispersant as well as dispersed ANS. Two oil degrading cultures, isolated from the surface (meso) and deep sea (cryo) of the Gulf of Mexico, were enriched on ANS crude oil at 25 °C (meso) and 5 °C (cryo) were used as inocula. Time series concentrations for the oil components (alkanes and aromatics) and an anionic surfactant in Finasol were determined. Results indicated that almost all the surfactant was biodegraded by meso culture at 25 C, but the surfactant was stable in the presence of the meso culture at 5 C and cryo cultures at 25 C and 5 C. Over 90% of the total alkane fraction was biodegraded for the oil with and without dispersant at both temperatures. For the aromatic fraction, the cryo culture metabolized 76% of the aromatics in ANS alone and 64% in ANS dispersed with Finasol, whereas aromatics persisted in both oil alone and dispersed oil samples at 25 °C. The results shed light on the effect of dispersant on the fate of spilled oil and rates of oil and dispersant biodegradation, which will be essential for dispersant usage.
- **Tracking and Planning**
  - ☐ Task: null
  - ☐ Product:
  - ☐ Project: Environmental Releases of Oil & Fuels
  - ☐ Science Question:
  - ☐ Topic:
  - ☐ Theme: Sustainable Approaches for Contaminated Sites and Materials Management
  - ☐ Research Program Area: Sustainable and Healthy Communities
- **HISA? ISI? High Profile?:** Not Applicable
- **QA form attached in STICS?:** Not Applicable
- **QAPP Reference:** N/A
- **Keywords:**
  - ☐ Biodegradation

- ☐ Oil Spills
- ☐ Finasol OSR 52
- ☐ ANS Crude Oil
- **Meeting Information:**
  - ☐ Meeting Name: 2016 Gulf of Mexico Oil Spill & Ecosystem Science Conference
  - ☐ Meeting Start Date: 02/01/2016
  - ☐ Meeting End Date: 02/04/2016
- **DOI:** <http://dx.doi.org/>
- **URL:** <http://www.cvent.com/events/2016-oil-spill-and-ecosystem-science-conference/event-summary-52ad0b225ba54cf0960090070e6f8073.aspx>

This submission can be found in the History tab. [Please click here to access STICS.](#)

**To:** Conmy, Robyn[Conmy.Robyn@epa.gov]  
**From:** Brooks, Rebecca J LT  
**Sent:** Tue 2/23/2016 5:49:44 PM  
**Subject:** FW: Visit to DC by the PWSRCAC  
[Signed Final Minutes-ICCOPR&PWSRCAC meeting 27MAR2014.pdf](#)  
[Final Minutes - PWSRCAC-ICCOPR Mtg 26MAR15 signed.pdf](#)  
[Invitation ICCOPR-PWSRCAC Mtg 26MAR2015.pdf](#)

Good morning Robyn,

We received a request from the Prince William Sound Regional Citizens' Advisory Council to coordinate a meeting on March 16. It appears as though they have come to DC annually in March to meet with members of the ICCOPR (I saw that you attended, according to the minutes, at least the past two meetings).

I have attached minutes and a meeting invite from the last couple of annual meetings we've had with them for your convenience. I am wondering if you'd like to participate and also if you have some thoughts on who all / who else we ought to invite -- does an invite go out to the whole ICCOPR membership or just a few folks? And please let me know if you'd like to see anything in particular on the agenda! Otherwise I think I'll keep it primarily to updates with goings-on in ICCOPR... and get an idea of their updates as well. They would also like a brief from NPFC which I can coordinate.

Thanks for your time and have a great day!

Very respectfully,

LT Becca Brooks  
U.S. Coast Guard Headquarters STOP 7516  
Office of Marine Environmental Response Policy (CG-MER-3)  
2703 Martin Luther King Jr. Ave SE  
Washington, DC 20593-7516  
Phone: 202-372-2259

-----Original Message-----

From: Roy Jones [mailto:rjones@sjgdc.com]  
Sent: Tuesday, February 23, 2016 9:56 AM  
To: Weaver, James D CDR  
Cc: Loring, Joseph B CAPT; Brooks, Rebecca J LT; Calhoun, Scott R CDR  
Subject: [Non-DoD Source] RE: Visit to DC by the PWSRCAC

Sounds great..thank you for coordinating this...and I look forward to hearing from Lt. Brooks and seeing the iccopr folks on the 16th....best...R.J.

Sent from my Verizon Wireless 4G LTE smartphone

----- Original message -----

From: "Weaver, James D CDR" <James.D.Weaver@uscg.mil>  
Date: 2/23/2016 9:38 AM (GMT-05:00)  
To: Roy Jones <rjones@sjgdc.com>  
Cc: "Loring, Joseph B CAPT" <Joseph.B.Loring@uscg.mil>, "Brooks, Rebecca J LT" <Rebecca.J.Brooks@uscg.mil>, "Calhoun, Scott R CDR" <Scott.R.Calhoun@uscg.mil>  
Subject: RE: Visit to DC by the PWSRCAC

Good Morning Mr. Jones -

The afternoon of March 16 at 1101 Pa. Ave should work. We'll reach out to other ICCOPR members for availability as well. LT Brooks will contact you to coordinate meeting time and presentation materials. Thank you and look forward to the meeting.

V/R,  
CDR James Weaver  
U.S. Coast Guard Headquarters STOP 7516  
Office of Marine Environmental Response Policy Chief, Interagency Coordination Division (CG-MER-3)  
2703 Martin Luther King Jr. Ave SE  
Washington, DC 20593-7516  
Phone: 202-372-2247

-----Original Message-----

From: Roy Jones [mailto:rjones@sjgdc.com]  
Sent: Monday, February 22, 2016 10:37 AM  
To: Loring, Joseph B CAPT; Calhoun, Scott R CDR; Weaver, James D CDR  
Cc: Wallin, Thomas W CDR; Brooks, Rebecca J LT  
Subject: [Non-DoD Source] RE: Visit to DC by the PWSRCAC

Good morning....and thank you for the update re: Bill V. and a designated hitter, CDR Weaver.

CDR Weaver-in past years we have met in DC with ICCOPR reps so as to facilitate people attending from various agencies. Last couple of years we did it at our office building at 1101 Pennsylvania Avenue, NW...or other downtown location...and we can do that again downtown if it makes sense and helps make it more likely that folks from several agencies can attend.

The dynamic of having NOAA, EPA, USCG, DOI and other agency reps. attend has been most helpful. And, it is a way too for the PWSRCAC to know what research is going on through ICCOPR and for ICCOPR to know what the PWSRCAC is doing....hopefully so that such research is not duplicated...and can be complementary to other's research in the field.

My suggestion is to plan that session early afternoon at 1101 Pa. Ave. ....say 1:30 p.m. - 3:00 p.m. or so. That way the group can meet and share info....and still let people get back to offices before rush hour.

CDR Wallin-the PWSRCAC will appreciate your arranging a meeting at USCG Hqs. with your folks who are on the front lines in Oil Pollution Prevention and Response....the RCAC is on the front lines also in terms of monitoring what is going on vis a vis Prince William Sound with the terminal and tanker traffic but depends as the public does for the CG to help make sure bad things don't happen.

In the aftermath of the 1989 spill as you know, something had to change to help ensure this type of accident didn't happen again. So far, so good. But it is a matter of "constant vigilance"...and these

citizens who make up the panel and come from all over the spectrum in the Exxon Valdez Oil Spill region take what they do very seriously....they saw what devastation can occur when government, industry and the public don't stay watchful.

Attached are short bios of the 4 people who will be traveling next month to DC and meet with Members of Congress, congressional staffs, the USCG, ICCOPR, et. al. I would suggest if it is possible to arrange a meeting with your folks on oil pollution prevention and response that we do that one in the morning, maybe 10:00 a.m. to 11:30 a.m. or so...which would give us time to discuss topics of interest to the RCAC and to your folks without encroaching on the lunch hour schedule.

One thought here would be to have someone from the OSLTF join the group for that meeting so that the RCAC representatives can hear a bit of an update on where things stand on the OSTLTF....its re-authorization....its cost of living indexing on liability...and other relevant issues.

I mentioned to CDR Calhoun that some years back the OSLTF was running out of funds....I was helping not only the PWSRCAC in whose interest on behalf of the public is to ensure that adequate funds are in that Fund....and the World Wildlife Fund who also found it in their best interest that the OSLTF be fully funded. Well, turned out that old friend Sen. Ted Stevens was a highly receptive audience....and believe too that the funding mechanism needed to be turned back on....(the 5 cents a barrel fee at the refinery)....well, having the likes of Sen. S., Sen. Lisa Murkowski and Rep. Don Young and others pushing....it got done....much to everyone's relief. They saw the whole 1989 spill happen and knew that much has to be done not only in Alaska but around the nation to do what can be done to prevent oil spills and to respond to them if they do occur in spite of best intentions.

So, you have lots of supporters for the OSLTF at the PWSRCAC.

Thank you all for trying to work the PWSRCAC folks into your schedules and arrange for appropriate people to attend. These sessions have always seem to be most helpful in the RCAC carrying out its statutory mission.

Best...

Roy

P.S. I am an old Vietnam vet Army guy...who planned a trip as Counsel to a Congressional Committee that took a congressional delegation into Puerto Rico and the Virgin Islands immediately after Hurricane Hugo. The committee had jurisdiction over both insular areas. It reminded me in terms of what we saw then in places to what it was like after battles in Vietnam. Almost total destruction. For the V.I., were it not for a Coast Guard ship and personnel arriving soon after the hurricane, the lawlessness would have likely been worse. Your folks and their mere presence made a huge difference. And, having worked for

so many years with Alaska Natives who live in outlying areas of Alaska, I know too that the USCG has oftentimes meant the difference between survival and not. So, you have a big fan here ... as you do with the PWSRCAC!! rj

202 536 4395

571 217 4347

-----Original Message-----

From: Loring, Joseph B CAPT [mailto:Joseph.B.Loring@uscg.mil]

Sent: Monday, February 22, 2016 7:05 AM

To: Calhoun, Scott R CDR; Roy Jones; Weaver, James D CDR

Cc: Wallin, Thomas W CDR; Brooks, Rebecca J LT

Subject: RE: Visit to DC by the PWSRCAC

Roy,

My apologies for the struggle to contact us. Bill did leave ICCOPR and now works for BSEE. CDR James Weaver can facilitate meetings on 16Mar. Looking forward to meeting with you.

Thanks

JBL

CAPT Joe Loring

U.S. Coast Guard

Office of Marine Environmental Response

Phone: 202-372-2231

Joseph.b.loring@uscg.mil <mailto:Joseph.b.loring@uscg.mil>

-----Original Message-----

From: Calhoun, Scott R CDR

Sent: Friday, February 19, 2016 6:34 PM

To: Roy Jones

Cc: Wallin, Thomas W CDR; Loring, Joseph B CAPT

Subject: RE: Visit to DC by the PWSRCAC

Roy - thank you for reaching out. I enjoyed speaking with you!

As discussed, RDML Thomas is not in town that week; however, I can get you in touch with some other Officers who are helpful.

CDR Wallin is a good POC to arrange with meeting with the folks that deal with oil pollution prevention and response. I copied him on this e-mail.

I recommend you contact CAPT Loring about ICCOPR and the OSLTF. He is also copied.

I am happy to help coordinate, but am confident that CDR Wallin and CAPT Loring are best able to assist.

Sincerely, Scott



-----Original Message-----

From: Roy Jones [mailto:rjones@sjgdc.com <mailto:rjones@sjgdc.com> ]

Sent: Wednesday, February 17, 2016 5:36 PM

To: Calhoun, Scott R CDR

Subject: [Non-DoD Source] Visit to DC by the PWSRCAC

Thank you for your call back. I was having a hard time raising anyone at USCG Hqs. today..was beginning to wonder if the phone numbers were off..so was so glad to hear you when you called back.

The group that I help here in DC is the Prince William Sound Regional Citizens' Advisory Council (PWSRCAC). They were established pursuant to OPA 90.

They are charged with monitoring responsibilities in an effort to help prevent another major oil spill as Alaska experienced in 1989. Their work is advisory only. But, they represent the interests of the public in matters that are key to, as a former 17th District USCG Commander put it, "keeping oil out of the water."

They will make their annual trek back here on March 15 and 16. The 15th will be taken up with visits on Capitol Hill primarily.and March 16 is the target date for meetings with USCG and with ICCOPR.

If you could please let me know who the best contact would be at CG Hqs. for ICCOPR.I put a call into Bill Vocke (our contact in the past with ICCOPR) but have not heard back.and was concerned that he may no longer be working

on ICCOPR matters or traveling, etc..

And, as I mentioned, if you could please let me know who I might talk with at the OSLTF, that would be helpful too. Keeping that fund healthy is a key goal of the PWSRCAC as it is with USCG.

Thank you for your assistance in lining up appropriate folks from the USCG to meet with the RCAC. I will send to you tomorrow several topics that they plan to discuss while in DC this time around.

Best.

Roy Jones

Consultant

Roy Stapleton Jones, Jr., Esq.

1101 Pennsylvania Avenue, N.W.

Sixth Floor

Washington, DC 20004

Tel: (202) 536-4395

Mobile: (571) 217-4347

Fax: (866) 615-0356

**To:** Medley, Lori[lori.medley@bsee.gov]; steve.lehmann@noaa.gov[steve.lehmann@noaa.gov]; Conmy, Robyn[Conmy.Robyn@epa.gov]; Melchert, Elena[Elena.Melchert@hq.doe.gov]; Weaver, James (USCG)[James.D.Weaver@uscg.mil]; Vocke, William CIV[William.T.Vocke@uscg.mil]  
**From:** Vocke, William CIV  
**Sent:** Wed 7/1/2015 7:37:41 PM  
**Subject:** R&T Plan Chapter 7 for WG  
Final Ch 7 for WG approval 2015-06-23 - track change version.docx  
Final Ch 7 for WG approval 2015-06-23.docx

All,

Attached is the edited Chapter 7 based on member comments and UNH input. This is the version I will put in the compiled plan. If you see any problems with it, please let me know so I can correct it before combining all the chapters.

The process I'm using to finalize the plan is to edit each chapter, send the edited version to the WG to give you a chance to see the edits, then add the individual chapters to a compiled R&T Plan to send to the members. Don't worry if you do not have time to look at these chapters as I send them -- we can always make changes on the compiled version.

Here is the current status of the plan chapters:

Ch 1 - adjudicated comments, sent to WG for comments (response from Lori)  
Ch 2&3 - Rewritten based on comments/input sent to members for final review  
Ch 4 - adjudicated comments. Waiting to reconcile Ch 4 and final Ch 9 SRA descriptions  
Ch 5&6 - Edits being finalized  
Ch 7&8 - Finalized edits sent to WG. Ready to add to compiled R&T Plan file  
Ch 9 - Adjudicated comments. Sent to WG for decision on text of priorities and SRA descriptions (response from Lori)

Have a great weekend,

Bill  
(202) 372-2019

**To:** Lori.Medley@bsee.gov[Lori.Medley@bsee.gov];  
steve.lehmann@noaa.gov[steve.lehmann@noaa.gov]; Conmy, Robyn[Conmy.Robyn@epa.gov];  
Elena.Melchert@hq.doe.gov[Elena.Melchert@hq.doe.gov]; Thompson, Sara  
LT[Sara.Thompson@uscg.mil]; Weaver, James (USCG)[James.D.Weaver@uscg.mil]  
**From:** Vocke, William CIV  
**Sent:** Thur 4/30/2015 7:59:32 PM  
**Subject:** ICCOPR R&T Plan  
Pt2 Ch 7&8 30APR15 Workgroup review draft.docx

Workgroup,

Thank you all for the comments on the other chapters. We are getting into the home stretch.

Attached for your review is a draft of Chapters 7 and 8 of the R&T Plan. Please review and provide me comments/edits by COB May 8, 2015. Then I will send out to the membership for comment.

In Chapter 7, we describe several spills and then list the SRAs and research needs the event revealed. Please give me your opinion on whether this is too much information. We could shorten this to only list the SRAs and not specific needs.

Thanks for your reviews.

Bill  
(202) 372-2019

**To:** Matthiessen, Craig[Matthiessen.Craig@epa.gov]; Wilson, Gregory[Wilson.Gregory@epa.gov]; Principe, Vanessa[Principe.Vanessa@epa.gov]; Conmy, Robyn[Conmy.Robyn@epa.gov]  
**From:** Barron, Mace  
**Sent:** Mon 4/13/2015 5:37:50 PM  
**Subject:** Re: FYI - : BP dispersant toxic

.....  
>>>>>>

Hi Craig: I read the summary below and the abstract, but don't have access to the paper.

Should I request through interlibrary loan from our librarian, or do you have a copy?

PS: Michael is retired, so I took him off this email.

Mace

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**From:** Matthiessen, Craig  
**Sent:** Monday, April 13, 2015 12:31 PM  
**To:** Wilson, Gregory; Principe, Vanessa; Conmy, Robyn; Barron, Mace; Hemmer, Michael  
**Subject:** FW: FYI - : BP dispersant toxic  
Any thoughts? Thanks - Craig

**From:** Nichols, Nick  
**Sent:** Monday, April 13, 2015 10:16 AM  
**To:** Matthiessen, Craig; Oliveira, Beatriz; DeHaven, Leigh; Gioffre, Patricia; Howard, MarkW; Swackhammer, J-Troy  
**Subject:** FYI - : BP dispersant toxic

FYI

**OCEANS:**

**Gulf spill cleanup dispersant more toxic to coral than oil -- study**

Katherine Ling, E&E reporter

Published: Friday, April 10, 2015

The chemical dispersant used to break down the oil spilled in the 2010 Deepwater Horizon accident in the Gulf of Mexico is more toxic to coral than the oil, according to a study released on the eve of the fifth anniversary of the disaster.

The accident marked the first time that a dispersant was used below the ocean surface during an oil spill in addition to the traditional surface use. Almost 2 million gallons of the

dispersant Corexit 9500A -- with slightly less than half released underwater -- was used to emulsify the around 5 million barrels of crude oil that poured out due to the rig explosion.

When exposed to the chemical dispersant, three species of cold-water coral showed "more severe health declines" at lower concentrations than the same species exposed to a mixture of oil and dispersant and to just oil, according to findings by scientists from Temple University and Pennsylvania State University. The oil-dispersant mixture was also more toxic than just oil, the team found.

Its study was published online in the journal *Deep-Sea Research II*.

The scientists became interested in the experiment after observing post-spill that several damaged Gulf coral populations were coated with a dark-colored wool-like slime that was found to contain oil from the spill and residues from the dispersants.

"We wanted to know if the damages that had been witnessed could have been caused by the oil, the dispersant itself, or a combination of both," Danielle DeLeo, a Temple doctoral student who was the study's lead author, said in a statement.

"We know that the corals in the Gulf were exposed to all of these different combinations, so we have been trying to determine the toxicity of the oil and the dispersants, and see what their impact would be on the corals," she said.

The corals may have a higher tolerance for the oil because they have adapted to natural seeps of oil over time in their environment, the team noted. The study also said that despite the results, "it is unclear whether short-term exposures to oil and dispersant have long-term effects," that the matter would require further study, and that long-term oil exposure could have significant sub-lethal impacts.

"Applying the dispersants at depth was a grand experiment being conducted in real-time," Erik Cordes, an associate professor of biology at Temple, said in a statement. He has been studying Gulf of Mexico coral communities for more than a decade.

He added: "It was a desire to immediately do something about the oil coming out of the well, but they really didn't know what was going to happen as a result."

The team concluded that to improve future oil spill response efforts, "alternative methods of oil cleanup are needed and caution should be used when applying oil dispersants at depth, as it may induce further stress and damage to deep-sea ecosystems."

The study was funded by the Gulf of Mexico Research Initiative, an independent agency BP PLC funded at \$500 million for 10 years in the wake of the Deepwater Horizon disaster that explores the impacts of oil spills and dispersants on local ecosystems, as well as developing improved spill mitigation, oil and gas detection, and other technologies.

Another recent study from the University of Alabama, Birmingham, found that the Corexit 9500A dispersant can damage respiratory cells of humans and animals. But a study by U.S. EPA in 2010 found the dispersant-oil combination isn't worse for shrimp, fish and other sea creatures than oil alone already is.

EPA announced a proposal to update regulations for the use of dispersants earlier this year. The plan would provide a new, well-tested and peer-reviewed laboratory method for gauging the effectiveness of products in different environments and an aquatic toxicity threshold to ensure qualifying products offer "greater performance at less environmental impact" (**Greenwire**, Jan. 13).

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ED 001324 00000039-00001





And a link to the study paper:

<http://www.sciencedirect.com/science/article/pii/S0967064515000740>

Looks like you'd have to purchase it. However, the EPA library system may be able to get it.

And I forgot Michael retired. Thanks - Craig

**From:** Barron, Mace

**Sent:** Monday, April 13, 2015 1:38 PM

**To:** Matthiessen, Craig; Wilson, Gregory; Principe, Vanessa; Conmy, Robyn

**Subject:** Re: FYI - : BP dispersant toxic

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